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MASOUD ISANEJAD

**NUTRITION AND MUSCULOSKELETAL
HEALTH AMONG OLDER PEOPLE**

*Nutrition and musculoskeletal health among
older people*

MASOUD ISANEJAD

*Nutrition and musculoskeletal health among
older people*

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ABSTRACT

Sarcopenia is characterized by a decline in skeletal muscle mass and muscle strength in the older individuals. It has been suggested that increased dietary protein intake and a healthy diet can prevent or delay the onset of sarcopenia. The aim of this thesis was to evaluate the association of protein intake with bone mineral density (BMD) and bone mineral content (BMC) (*Study I*) in the Osteoporosis Risk Factor and Prevention–Fracture Prevention Study, a cohort of Finnish elderly women, $n=554$, aged 65–72 years. This thesis also examined the associations of intakes of total protein, animal protein and plant protein with changes in muscle mass (*Study II*), and muscle strength and physical function at baseline and over 3 years of follow-up (*Study III*). We also evaluated whether women with a protein intake higher than 1.1 g/kg body weight and who were not obese would have a lower odds ratio of frailty (*Study IV*). Finally, we investigated the association of the Baltic Sea diet (BSD) and Mediterranean dietary patterns (MED) with indices of sarcopenia (*Study V*).

After adjustment for confounders, dietary energy-adjusted intakes of total and animal protein (g/d), but not plant protein, were negatively associated with femoral neck BMD and BMC (*Study I*). Furthermore, women with a higher protein intake i.e. ≥ 1.2 g/kg body weight, had lower femoral neck, lumbar spine and total BMD and BMC. Women in the higher quartiles of total and animal protein intake exhibited less muscle mass loss (*Study II*), the association was more pronounced among weight maintainers. A dietary protein intake ≥ 1.1 g/kg body weight and a lower body fat mass were positively associated with muscle strength and physical function in elderly women (*Study III*). Subjects with protein intake ≥ 1.1 g/kg BW had a lower risk of prefrailty ($n=206$) (OR=0.08 and 95% confidence interval=0.01-0.73) and frailty ($n=36$) (OR=0.08 and CI=0.01-0.72) compared to those with protein intake <1.1 g/kg BW (*Study IV*). Furthermore, obesity (BMI ≥ 30 kg/m²) was associated with prefrailty (OR=2.81 and CI=1.47-5.37) and frailty status (OR=4.72 and confidence interval=1.26-17.60), but this was not the case for overweight (BMI 25 to <30 kg/m²). Finally, this study showed that women in the higher quartiles of BSD and MED scores lost less muscle mass (*Study V*). Women with higher concordance to BSD and MED had a faster 10 m walking speed, and a better lower body muscle quality (10 m walking speed/leg muscle mass). Taken together, an increase in dietary protein intake and concordance to BSD and MED may be associated with a reduced risk of sarcopenia.

National Library of Medicine Classification: QT 235, QT 256, QU 55.4, WE 202, WE 504

Medical Subject Headings: Diet; Dietary Proteins; Sarcopenia; Muscles; Muscle Strength; Adipose Tissue; Body Weight; Obesity; Physical Fitness; Osteoporosis; Bone and Bones; Bone Density; Frailty; Women; Female; Aged; Finland

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Ravitsemuksen vaikutuslihaksiston ja luuston terveyteen ikääntyneillä

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TIIVISTELMÄ

Sarkopeniaan liittyy lihasmassan ja lihasvoiman heikkenemistä ikääntyneillä. Sarkopenia lisää alttiutta osteoporoosiin, murtumiin, heikentyneeseen elämänlaatuun, vammoihin ja kuolleisuuteen. On esitetty, että suurempi ruokavalion proteiinin saanti ja terveellinen ruokavalio voisivat estää tai myöhästyttää sarkopenian alkamista. Tämän tutkimuksen tavoite oli arvioida ruokavalion proteiinin saannin yhteyttä luun mineraalitiheyteen ja –pitoisuuteen (Osatyö I) Kuopion Osteoporoosin Vaaratekijät ja Ehkäisy – Murtuman ehkäisy (OSTPRE-FPS) tutkimuksessa ikääntyneillä naisilla (n=554, 65-72 vuotta). Tutkimuksessa tarkasteltiin myös proteiinin sekä eläinkunnan ja kasvikunnan proteiinin yhteyttä lihasmassan (Osatyö 2), lihasvoiman ja toimintakyvyn (Osatyö 3) muutoksiin kolmen vuoden seurannassa. Tutkimme myös, onko naisilla, joiden proteiinin saanti on suurempaa kuin 1,1 g/kg kehonpaino ja jotka eivät olleet lihavia, pienempi gerastenian riski. Viimeinen tavoite oli tutkia Itämeren ja Välimeren ruokavalioiden yhteyttä sarkopenian osatekijöihin.

Ruokavalion energiaan suhteutettu proteiinin ja eläinkunnan proteiinin saannit olivat käänteisesti yhteydessä reisiluun kaulan mineraalitiheyteen ja –pitoisuuteen, kun sekoittavat tekijät oli vakioitu (Osatyö I). Lisäksi naisilla, joilla proteiinin saanti oli $\geq 1,2$ g/kg kehonpaino, oli pienempi reisiluun kaulan, lannerangan ja kokonaisluuston mineraalitiheys ja –pitoisuus. Naisilla, joilla proteiinin ja eläinkunnan proteiinin saannit olivat suurimmissa kvartileissa, oli vähemmän lihaskatoa (Osatyö II). Tämä yhteys oli voimakkaampi henkilöillä, joilla kehonpaino ei muuttunut. Proteiinin saanti $\geq 1,1$ g/kg kehonpaino ja pienempi kehon rasvamassa olivat suoraan yhteydessä lihasvoimaan ja toimintakykyyn ikääntyneillä naisilla (Osatyö III). Naisilla, joilla proteiinin saanti oli $\geq 1,1$ g/kg kehonpaino, oli pienempi gerastenian esiasteen (n=206, riskisuhde 0,08, 95% luottamusväli 0,01-0,73) ja gerastenian (n=36, riskisuhde 0,08, 95% luottamusväli 0,01-0,72) riski verrattuna naisiin, joiden proteiinin saanti oli vähäisempää (Osatyö IV). Lisäksi lihavuus (kehon painoindeksi ≥ 30 kg/m²), mutta ylipaino (kehon painoindeksi 25-30 kg/m²), oli yhteydessä gerastenian esiasteeseen (riskisuhde 2,82; 95% luottamusväli 1,47-5,37) ja gerasteniaan (riskisuhde 4,72; 95% luottamusväli 1,26-17,60). Lopuksi tässä tutkimuksessa osoitettiin, että naiset, joilla Itämeren ja Välimeren ruokavalion noudattamista kuvaavat indeksit kuuluivat suurimpiin kvartileihin, menettivät vähemmän lihasmassaa (Osatyö V). Naisilla, jotka noudattivat eniten Itämeren tai Välimeren ruokavaliota, oli nopeampi 10 m kävelyvauhti, parempi alavartalon lihasten (10 m kävelyvauhti/jalkojen lihasmassa). Yhteenvetona voidaan todeta, että suurempi proteiinin saanti ruokavaliosta ja Itämeren tai Välimeren ruokavalion noudattaminen voivat olla yhteydessä pienempään sarkopenian riskiin.

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Yleinen suomalainen asiasanasto: ravitsemus; ruokavaliot; proteiinit; lihakset; lihasvoima; lihasmassa; rasvakudokset; lihavuus; fyysinen toimintakyky; osteoporoosi; luusto; luuntiheys; naiset; ikääntyneet; Suomi

"My knowledge got this far: enough to know that I know nothing!"

Bu Ali Sina (بو علی سینا) 980-1037

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- I. Isanejad M, Mursu J, Sirola J, Kröger H, Tuppurainen M, Erkkilä A.T. Association of protein intake with bone mineral density and bone mineral content among elderly women: the Ostpre Fracture Prevention Study. *Journal of Nutrition, Health and Aging* 21 (6): 1-9, 2017.
- II. Isanejad M, Mursu J, Sirola J, Kröger H, Tuppurainen M, Rikkinen T, and Erkkilä A.T. Association of protein intake with the change of lean mass among elderly women: The Osteoporosis Risk Factor and Prevention–Fracture Prevention Study (OSTPRE-FPS). *Journal of Nutritional Science* 4 (41): 1-8, 2015.
- III. Isanejad M, Mursu J, Sirola J, Kröger H, Tuppurainen M, Rikkinen T, Erkkilä A.T. Dietary protein intake is associated with better physical function and muscle strength among elderly women. *British Journal of Nutrition* 115, 1281-1291, 2016.
- IV. Isanejad M, Rikkinen T, Sirola J, Mursu J, Kröger H, Qazi S.L, Tuppurainen M, Erkkilä A.T. Association of Dietary Protein Intake and Obesity with Frailty Status in Elderly Women. *Submitted*.
- V. Isanejad M, Mursu J, Sirola J, Kröger H, Tuppurainen M, Rikkinen T, Erkkilä A.T. Association of the Baltic Sea and Mediterranean diets with indices of sarcopenia in elderly women, OSPTRE-FPS study. *European Journal of Nutrition*, 10.1007/s00394-017-1422-2, 2017.

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Abbreviations

aLM	appendicular lean mass	LS	Lumbar spine
		MED	Mediterranean diet
BMC	Bone mineral content	MM	Muscle mass
BMD	Bone mineral density	mTORC1	Mammalian target of rapamycin
BMI	Body mass index		
BSD	Baltic Sea diet	NNR	Nordic nutrition recommendation
BW	Body weight		
DXA	Dual-energy X-ray absorptiometry	OSTPRE	Osteoporosis Risk Factor and Prevention
EWGSOP	European Working Group on Sarcopenia in Older People	OSTPRE-FPS	Osteoporosis Risk Factor and Fracture Prevention Study
FM	Fat mass	RSMI	Relative skeletal muscle index
FN	Femoral neck		
IGF	Insulin-like growth factor	SPPB	Short physical performance battery
LBMQ	Lower body muscle quality	WHO	World Health Organization
LM	Lean mass		
LMI	Lean mass index		

1 Introduction

The European population is undergoing an unprecedented ageing process, which is the consequence of the joint effects of increased life expectancy and reduced mortality. The percentage of people aged 60 years and older increased from 9% in 1990 to 11% in 2013 and it is expected to reach 21% by 2050 (Rafalimanana and Lai 2013). Due to increased life expectancy and the number of years lived, the health status of the elderly and their health care needs have radically transformed. Healthy aging defined as “the process of developing and maintaining the functional ability that enables well-being in older age” (Briggs et al. 2016) has received the attention of researchers. Thus, the preservation of the capacity to live independently and function well in the growing older European population poses an unprecedented public health challenge.

It is well known that sarcopenia and frailty are two major geriatric conditions posing a risk that older persons will develop adverse health outcomes such as fractures, metabolic syndrome, loss of independence, institutionalization or mortality (Cesari et al. 2014a, Nowson and O'Connell 2015). It has been estimated that the direct health care cost attributable to sarcopenia in the United States in 2000 was \$18.5 billion (Janssen et al. 2004). Although definitions and estimates of prevalence vary, sarcopenia is widely recognized as a common condition among older adults.

Although there is no consensus definition of frailty, it is agreed that frailty is mainly observable as diminished physical strength and endurance in the older individuals. The Fried frailty index classifies frailty as the presence of three or more of the following five components: weight loss, exhaustion, weakness, slowness and low physical activity (Fried et al. 2001). In a recent systematic review examining 61,500 community dwelling adults aged 65 and older, the overall

prevalence of frailty was estimated to be 10.7 %, and 41.6% were pre-frail (presence of one or two components of the Fried frailty) (Collard et al. 2012). Nevertheless, because of the diverse definitions of frailty status used in those studies, the reported prevalence varies extensively, ranging from 4.0–59.1 %.

Older adults have a propensity to eat less and it has been reported that the food intake is reduced by around 25% after 70 years of age (Nieuwenhuizen et al. 2010a, Suominen et al. 2014). In addition, due to a range of physiological, psychological, and social factors, in comparison with younger adults, elderly individuals eat more slowly, and they feel less hungry and thirsty, which means that they eat smaller meals and snacks (Nieuwenhuizen et al. 2010a). Previous studies, although limited, have shown that a healthy diet and regular physical activity are effective tools for extending healthy aging and preventing mobility-related disability. Declining muscle strength and physical capability may increase the risk of poor nutrition and conversely poor nutrition may contribute to further declines in physical capability. Preventing sarcopenia and frailty in the older individuals, via lifestyle approaches such as nutrition and physical activity, are of increasing research and public health interest.

There is accumulating evidence suggesting that an increase in protein intake can promote optimal health, preserve MM and physical function in the older individuals (Nordic Nutrition Recommendations 2013). The 2012 Nordic nutrition recommendations (NNR) highlighted the importance of sufficient protein intake in the maintenance of physical capacity in the older individuals (Beasley et al. 2010, Meng et al. 2009a, Gregorio et al. 2014). Furthermore, a dietary pattern approach rather than a single food or nutrient is attracting growing interest. Mediterranean (MED) and Baltic Sea Dietary (BSD) patterns have been explored regarding their potential beneficial role on chronic diseases, morbidity, longevity and Alzheimer's disease (Sofi et al. 2014, Ruusunen et al. 2013). A diet concordant with MED may improve the measures of lower extremity function in the older individuals (Zbeida et al. 2014). It has recently been

postulated that BSD is associated with better overall physical performance among Finnish elderly women (Perala et al. 2016). However, the role of diet in the prevention and treatment of sarcopenia and frailty has not been extensively studied in the older individuals.

The aim of this doctoral thesis was to explore the role of dietary protein in bone mineral density (BMD), sarcopenia and frailty in Finnish elderly women. We examined whether a higher protein intake would be associated with preserving bone mass, MM, maintaining good physical function, and lowering the prevalence of sarcopenia and frailty compared with the lower intake. We also explored the association of sources of protein intake, i.e. animal protein and plant protein, with MM, physical function, sarcopenia and frailty. Finally, another aim was also to assess the relationship between a healthy diet as indicated by BSD and MED dietary patterns with MM, physical function and sarcopenia in Finnish elderly women.

2 *Literature review*

2.1 **SARCOPENIA**

Sarcopenia is recognized as a major public health problem since it has significant clinical, economic, and social consequences (Beaudart et al. 2014b). The term ‘sarcopenia’ was coined for the first time by Irwin Rosenberg in 1989; it has the Greek root of ‘sarx’ meaning flesh and ‘penia’ meaning loss. Subsequently, sarcopenia has since been widely defined as a loss of skeletal MM that occurs with advancing age. Sarcopenia is associated with a decrement in strength, and consequently a higher risk of mobility disability and functional limitations in daily living activities (Bergerand Doherty 2010), an increased incidence of falls and fractures (Scott et al. 2016, Chalhoub et al. 2015). The decline in muscle strength during ageing was originally attributed to loss of MM but further studies revealed that muscle strength loss may outpace MM loss, thus its association with the functional decline can be independent of MM (Visserand Schaap 2011).

Several groups and committees have published operational criteria to define sarcopenia: (i) International Working Group (IWG) (Fielding et al. 2011) ; (ii) European Working Group on Sarcopenia in Older Persons (EWGSOP)(Cruz-Jentoft et al. 2010); (iii) Society of Sarcopenia, Cachexia, and Wasting Disorders (SIG) which was created within European Society for Clinical Nutrition and Metabolism Special Interest Group on cachexia-anorexia in chronic wasting diseases (ESPEN) (Morley et al. 2011, Muscaritoli et al. 2010); and (iv) Foundation for the National Institutes of Health (FNIH) Sarcopenia Project (Dam et al. 2014). The various proposed operational definitions and prevalence of sarcopenia are presented in Table 1.

Table 1. Summary of operational definitions for sarcopenia

Criteria	Operational Definition		
	Physical performance	Muscle Strength	Muscle Mass
FNIH	Slow walking speed: ≤ 0.8 m/s	Grip strength: men <26 kg, and women <16 kg	ALM _{BMI} : men <0.789 , and women <0.512 .
IWG	Slow walking speed: <0.8 m/s	not part of definition	ALM/ht ² : men ≤ 7.23 kg/m ² , and women ≤ 5.67 kg/m ²
EWGSOP	Slow walking speed: <0.8 m/s	Grip strength: men <30 kg, and women <20 kg	ALM/ht ² : men ≤ 7.23 kg/m ² , and women ≤ 5.67 kg/m ²
SIG	Slow walking speed: <0.8 m/s or replaced with another functional tests utilized locally.	not part of definition	a % of muscle mass 2 SD below the mean measured in young adults of the same sex and ethnic background.

FNIH, Foundation for the National Institutes of Health (FNIH) Sarcopenia Project; IWG, International Working Group on Sarcopenia; EWGSOP, European Working Group on Sarcopenia in Older Persons; SIG, Society of Sarcopenia, Cachexia, and Wasting Disorders ; ALM, appendicular lean mass.

At present, the EWGSOP definition has been most commonly used in epidemiologic studies; most of these studies have demonstrated an association between sarcopenia and functional decline, hospitalization, and mortality in both community dwelling older adults and residents in nursing homes (Maeda and Akagi 2016, Morley et al. 2014a, Rolland et al. 2008). In 2010, EWGSOP proposed a consensus definition to diagnose sarcopenia (Cruz-Jentoft et al. 2010, Cruz-Jentoft et al. 2014).

Other European scientific organizations (the European Society of Clinical Nutrition and Metabolism, the International Academy of Nutrition and Aging and the International Association of Gerontology and Geriatrics—European Region) were also involved in shaping the rationale and methods to define sarcopenia. EWGSOP recommends that sarcopenia can be diagnosed by the presence of both low MM and low muscle function (strength or performance) (Cruz-Jentoft et al. 2010). The emergence of muscle function criteria in the EWGSOP definition was due to their importance in the prediction of frailty and fracture (Lauretani et al. 2003, von

Haehling et al. 2010, Cruz-Jentoft et al. 2014), muscle strength does not depend solely on MM, and the relationship between strength and mass is not linear (Delmonico et al. 2009).

Additionally, sarcopenia may not simply be related to a decline in physical function and frailty but it can increase the risk of metabolic syndrome and chronic disease such as diabetes (Morley et al. 2014b). An increased body of evidence supported the importance of diagnosis and treatment of sarcopenia, therefore, as of October 1st, 2016, sarcopenia has received its own International Classification of Disease, Tenth revision (ICD-10-CM). The assigned code will be M62.84.

Further, EWGSOP suggested a category for staging sarcopenia which can reflect the severity of the condition, and can help guide in the clinical management of the condition. The different stages of sarcopenia are presented in Table 2 (Cruz-Jentoft et al. 2010). EWGSOP suggests a conceptual staging as ‘presarcopenia’, ‘sarcopenia’ and ‘severe sarcopenia’.

Table 2. EWGSOP conceptual stages of sarcopenia (Cruz-Jentoft et al. 2010)

Stage	Muscle mass	Muscle strength	Physical performance
Presarcopenia	↓	-	-
Sarcopenia	↓	↓	Or ↓
Severe sarcopenia	↓	↓	↓

The presarcopenia stage is the condition when low MM exists without any impact on muscle strength or physical performance. In order to identify this stage, the only way is to measure MM in reference to the standard population. However, several studies have reported that the comparison to the reference population is not always feasible because genetic and ethnicity factors affect the MM in different populations (Sjoblom et al. 2013, Isanejad et al. 2016). The ‘sarcopenia’ stage is characterized by low MM, plus low muscle strength or low physical

performance. 'Severe sarcopenia' is the stage identified when all three criteria of the definition are met (low MM, low muscle strength and low physical performance) (Cruz-Jentoft et al. 2010).

2.1.1 Pathophysiology of sarcopenia

There are multifactorial mechanisms involved in sarcopenia; these are associated with low physical activity level and sedentary lifestyles (Janssen et al. 2002), inadequate nutrition or malnutrition (Volkert 2011), and loss of anabolic and anticatabolic responsiveness to changes in extracellular amino acid concentrations (Katsanos et al. 2006), as well as a disturbed balance of oxidant and antioxidant defenses in the body (Kim et al. 2010) or other plausible cellular mechanisms. Although considerable progress has been made in recent years to identify the major contributors to sarcopenia, knowledge regarding the development of sarcopenia is scarce. It seems plausible that the main factor in sarcopenia development may be explained by the inability of the skeletal muscles to compensate for the muscle degenerative/deteriorative processes. This alteration impairs the myogenic mechanisms, responsible for maintenance of MM and muscle protein turnover (Petrella et al. 2006). Figure 1 provides a summary of the potential major contributors to sarcopenia, such as sex hormones, low grade inflammation, physical activity, nutrition etc.

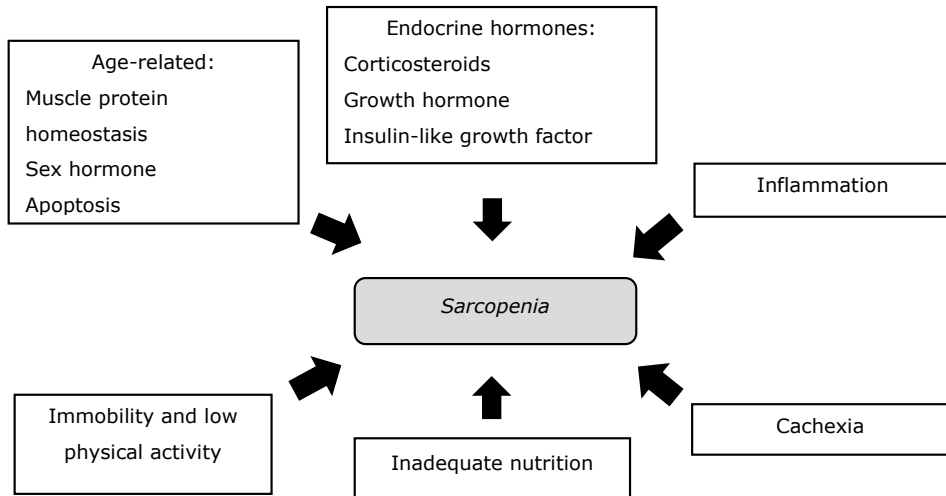


Figure 1. Multifactorial mechanisms underlying sarcopenia.

Inflammation and sarcopenia

Chronic low-grade inflammation, which is quite different from an acute inflammation process, plays an important role in age-related diseases. Inflammation is one potential pathophysiological phenomenon, which may be closely linked with MM loss, physical function decline, and bone mass loss in the older individuals. Proinflammatory cytokines (tumor necrosis factor [TNF]- α , interleukin [IL]-1 β and IL-6) might increase MM loss directly by decreasing protein synthesis and increasing myofibrillar protein degradation (Walrand et al. 2011). These proinflammatory factors are involved in the regulation of muscle protein turnover. In general, aging is accompanied by increased levels of circulating inflammatory markers in blood (Walrand et al. 2003). The low-grade inflammation may be associated with sarcopenia, osteoporosis, atherosclerosis, reduced immune function, and insulin resistance in the older individuals (Walrand et al. 2003).

The signaling pathways of protein synthesis and degradation are regulated by multiple factors. Dietary factors and obesity have been linked to the regulation of inflammatory factors in the body (Batsis et al. 2016, Boirie 2009). Higher concordance with the Alternate Healthy Eating Index was associated with a 21% lower likelihood of having high-risk C-reactive protein (Mattei et al. 2017). This might be one plausible explanation why diet and obesity are related to age-related muscle deterioration.

Age-related changes in hormone levels and sensitivity

Aging is associated with modifications in hormone production and sensitivity, especially with growth hormone, insulin-like growth factor (IGF)-1, corticosteroids, androgens, estrogens, and insulin. The functions of these hormones may influence optimal muscle protein metabolism. IGF-1 plays an important role in the regulation of growth and is known to exert anabolic effects on skeletal muscle that are important for normal body functioning (Pisciottano et al. 2014). The increased production of circulating IGF-1 is a response to increased levels of growth hormone; IGF-1 can be produced in various tissues, including skeletal muscle and bone (Nindl et al. 2010). It has been well documented that IGF-1 is strongly associated with the preservation of muscle body mass, therefore, its decline is thought to predispose older individuals to sarcopenia, and the loss of functional dependency (Lang et al. 2010, Arnarson et al. 2015). Accordingly, increased concentrations of IGF-1 have been associated with MM (Velloso 2008) which can be important in sarcopenia. However, more studies are required at the cellular level to explore the mechanisms through which IGF-1 can influence MM and function.

Age-related changes in muscle protein homeostasis

Skeletal muscle has a protein content in the range 50–75% and it is considered as the primary amino acid reservoir of human body (Cruz-Jentoft et al. 2012). It appears that a number of factors are responsible for the imbalance in protein breakdown and muscle production,

including increased anabolic resistance to protein and amino acids feeding, impairment of protein synthesis and increased protein breakdown in the older people.

Aging is accompanied by an impaired ability to activate mammalian target of rapamycin (mTORC1) signaling, which is crucial in muscle protein synthesis (Cruz-Jentoft et al. 2010, Eley et al. 2007). The declines in the efficiency of protein synthesis may be mediated through impairments in mTOR-dependent increases in translation initiation (Eley et al. 2007). mTORC1 regulates translation and cell growth by coordinating upstream inputs such as growth factors, intracellular energy status, and amino acid availability (Markofski et al. 2015, Cruz-Jentoft et al. 2012). The results of a limited number of studies have revealed that mTORC1 stimulates muscle protein synthesis in response to insulin, muscle contraction and nutrients (mainly essential amino acids) in humans (Drummond et al. 2009, Han et al. 2012). However, little data are available on the mTORC1 signal pathway as well as muscle protein synthesis in the older individuals and further studies are warranted.

Although the accurate measurement of muscle protein breakdown rate in vivo in humans is challenging, the current available data in humans imply that the older individuals seem to have slightly elevated rates of proteolysis in a fasting state compared to that in their younger counterparts (Burd et al. 2013). Furthermore, attenuation of post-absorptive muscle protein synthesis may underpin the gradual aging decline of MM (Short et al. 2004). Therefore, the decline in the response to an anabolic stimulus for skeletal muscle can be one of the main mechanisms in an age-related loss of MM and function (Burd et al. 2013).

The MM loss in the older individuals is mainly an imbalance between muscle protein synthesis and muscle protein breakdown. The MM loss which results in sarcopenia progresses gradually over decades. However, the evidence is inconclusive regarding the decline in muscle protein synthesis in older adults compared to their younger peers. The results of a recent study showed that age and sex did not influence basal muscle protein synthesis (Markofski et al. 2015), while

it was also reported that there is a decrease in the basal muscle protein synthesis rate with age (Balagopal et al. 1997). Thus, protein turnover is most likely disturbed due to alterations in other metabolic conditions, such as muscle contraction mechanisms or nutrient intakes (Cruz-Jentoft et al. 2012, Rolland et al. 2008).

Physical activity and sarcopenia

Physical inactivity is linked to losses of MM, a decline in physical function and increased risk of sarcopenia and frailty, thus physical activity plays an important role in sarcopenia. Studies have shown that even with aging, sarcopenia and alteration in muscle function, muscle fibers in older adults remain responsive to different functional demands such as physical exercise (Rolland et al. 2008, Pillard et al. 2011). Thus, it has been suggested that regular physical activity can partially prevent sarcopenia and the frailty progression related to inactivity. The influence of physical activity in sarcopenic muscle has been described in relation to several of the factors acting on muscle in age-related imbalance processes. There is a report that exercise can upregulate the mTORC1 pathway in animals and young humans (Pillard et al. 2011).

In the study of Mijnders et al. conducted in subjects aged 66–93 years (n= 2309), the activity level was assessed by a self-reported questionnaire (Mijnders et al. 2016). The incidence of sarcopenia over 5 years was 14.8% in the least-active (weekly, but <1 h/week) individuals and 9.0% in the most-active individuals (>4 h per week). Compared with the least-active participants, those reporting moderate to high amounts of moderate-vigorous physical activity (1–3 h per week) had a significantly lower likelihood of incident sarcopenia even when adjusted for potential confounders. Those participants with a high amount of moderate–vigorous physical activity (>4 h per week) had higher baseline levels of MM, strength and walking speed.

Cruz-Jentoft et al. (Cruz-Jentoft et al. 2014) published a systematic review in 2014 on the effect of physical activity and/or dietary supplementation on sarcopenia. The results indicated that most exercise trials showed an improvement of muscle strength and physical performance with

physical activity, predominantly in resistance training interventions. In another recent systematic review published by Beaudart et al. (2017), the combined effect of physical activity and dietary intervention (proteins, essential amino acids, creatine, β -hydroxy- β -methylbutyrate, vitamin D, multi-nutrients, or other) was summarized (Beaudart et al. 2017). The results indicated that physical exercise exerts a positive impact on MM and muscle function in healthy subjects aged 60 years and older. The largest effect of any type of exercise intervention was seen on physical performance (gait speed, chair rising test, and balance test), but the interaction with nutrition supplementation was inconclusive due to the huge variations in the dietary supplementation protocols.

2.1.2 Measurements of sarcopenia indices

Muscle mass

In order to measure MM, different body imaging techniques have been used, such as computed tomography, magnetic resonance imaging and dual-energy X-ray absorptiometry (DXA). Computed tomography and magnetic resonance imaging are considered to be very precise imaging systems that can separate fat from other soft tissues of the body. However, cost, limited access to equipment at some sites and concerns about radiation exposure all limit the use of these whole-body imaging methods for routine clinical practice (Chien et al. 2008, Cruz-Jentoft et al. 2010). DXA is a common alternative method both for research and clinical use to distinguish fat mass (FM), bone and lean mass (LM) (Chien et al. 2008).

Nevertheless, different indices of MM have been introduced for sarcopenia in the literature, including lean mass index (LMI) calculated by dividing MM by height (m), relative skeletal muscle index (RSMI), calculated by the sum of MM in arms and legs divided by height. Furthermore, an MM value 2 SD below the mean MM of young, adult reference population was defined as the cut-off point for sarcopenia (Baumgartner et al. 1999). Other studies have used population cut-off points such as the lowest 20 or 25 % of LMI or RSMI (Newman et al. 2003,

Sjoblom et al. 2013). Newman et al. (Newman et al. 2003) performed an observational cohort study of older people living in the USA (ages 70–79 years, $n = 2,984$). MM was assessed using DXA. Participants were classified as sarcopenic by using two different approaches to adjust lean mass (LM) to body size: appendicular lean mass (aLM) divided by height squared (aLM/ht^2) and aLM adjusted for height and body FM. In men, both classifications of sarcopenia were associated with negative health characteristics such as smoking, poorer health, lower activity and impaired lower extremity function. In women, the classification based on both height and FM adjustment was more strongly associated with impaired lower extremity function, while other associations were less strong. The authors suggested that fat mass should be considered in estimating the prevalence of sarcopenia in women and in overweight or obese individuals (Newman et al. 2003).

Muscle strength and physical performance

It has been noted that muscle strength may be a better predictor of disability than MM in older people (Studenski et al. 2014). There are several tests evaluating muscle function and performance which have been used in different studies to define sarcopenia or predict mobility disability, fall, fracture or morbidity (Martien et al. 2015, Cruz-Jentoft et al. 2010, Newman et al. 2003). For the quantification of muscle strength in older adults, dynamometric measures of handgrip and knee extension strength predominate (Bohannon et al. 2014). Isometric handgrip strength is clearly related with lower extremity muscle power and knee extension (Lauretani et al. 2003). Handgrip strength can be a useful tool for identifying a mobility limitation in clinical practice, and several studies have revealed a link between handgrip strength with mobility, daily activity, chronic disease and morbidity in the older individuals (Lawman et al. 2016, de Souza Vasconcelos, Karina Simone et al. 2016, Alley et al. 2014). Although the definition of low handgrip strength has remained controversial, a handheld dynamometer can be a reliable

measure of muscle strength, and it correlates with sarcopenia (Cruz-Jentoft et al. 2014, Studenski et al. 2014).

However, since aging is more associated with a decline in lower rather than upper body muscle size and strength, using grip strength to describe overall muscle strength and as a predictor of sarcopenia may not be appropriate (Bohannon et al. 2014). A wide range of tests of physical performance are available, although walking speed has received the most attention. It has been shown that walking speed displays a non-linear relationship with leg strength. EWGSOP has suggested that walking speed can be used in clinical and research settings, as well as in the diagnosis of sarcopenia (Cruz-Jentoft et al. 2010). Additionally, knee extensors in particular seem to be associated with several functional tests, such as walking speed, chair rising and stair climbing (Ploutz-Snyder et al. 2002). Knee extensor can be measured both isometrically or isokinetically, with the latter being a closer reflection of muscle function in everyday activities (Cruz-Jentoft et al. 2010). Martien et al. have examined whether knee extension strength is a better predictor of functional performance than handgrip strength among older adults ($n=770$, age ≥ 60 years) (Martien et al. 2015). Their results showed that both handgrip strength and knee extension strength (standardized for body weight) were positively correlated with functional performance (Buchner et al. 1996).

2.1.3 Sarcopenia screening and assessment

Prompt diagnosis of sarcopenia among community dwelling older people can detect those individuals at a higher risk for adverse outcomes, such as mobility disability, loss of independence, and increased risk for falls, and comorbidities at an earlier stage (Mijnarends et al. 2013). Multiple approaches and indices have been applied in different studies to assess sarcopenia as explained earlier (Bergerand Doherty 2010, Hedayatiand Dittmar 2010, Cruz-Jentoft et al. 2014). These definitional approaches can potentially use simple muscle strength tests (e.g., handgrip strength) or physical performance tests (e.g., walking speed, and standing

balance etc.,) as objective screening measures to identify those who might benefit from targeted interventions. However, the most commonly used methods to define sarcopenia in the European population have been based on the EWGSOP consensus definition. In 2010, EWGSOP proposed the use of an algorithm and assessment techniques to define sarcopenia in the elderly (Figure 2) (Cruz-Jentoft et al. 2010). The main parameters to define sarcopenia are the amount of MM and its function. Therefore, the measurable variables are MM, muscle strength and physical performance.

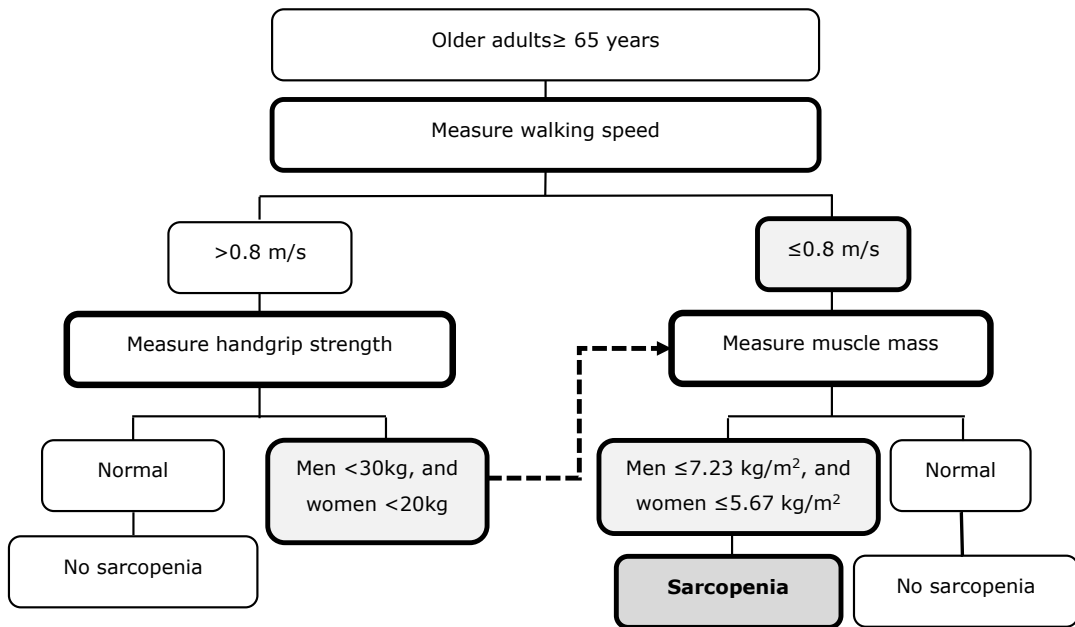


Figure 2. Algorithm suggested by European working group on sarcopenia to diagnose sarcopenia in older adults aged 65 years and older. The EWGSOP has offered three recommendations for identifying sarcopenia in clinical practice: (1) assess patient for slow walking speed indicated as ≤ 0.8 m/s. A cut-off point of <0.8 m/s identifies risk for sarcopenia (35), (2) identify patients with low handgrip strength, (3) consider sarcopenia in patients who have low walking speed and/or handgrip strength along with low muscle mass.

2.2 FRAILTY

2.2.1 Pathophysiology of frailty

Several pathophysiologic processes are linked to the development of frailty. A predominant role has been attributed to inflammatory mechanisms. As explained earlier, the pathways leading to inflammation and sarcopenia are considered to be similar. Although the role of inflammation is not clear in the pathogenesis of frailty, increased levels of C-reactive proteins and proinflammatory cytokines were significantly associated with the presence of frailty (Leng et al. 2007).

Changes in body composition are prevalent as an individual ages, especially a relative increase in fat mass and a loss of lean mass can be considered as important aspects in the pathogenesis of frailty. Data from the Cardiovascular Health Study showed that frail individuals were characterized by higher weight, more central obesity and a higher probability for exhibiting the metabolic syndrome (Barzilay et al. 2007).

Furthermore, obesity is common in aging and is known to be associated with disability and adverse health outcomes in the older individuals (Blaum et al. 2005). The results of a 22 year follow-up study in 1,119 men and women aged 30 or older, showed that those with obesity in midlife had more than a doubled risk for prefrailty and a five times higher risk for frailty later compared with their normal-weight peers and this was independent of age, sex, education, lifestyle factors, and chronic conditions. Compared to peers with BMI in the normal range (20-24.9 kg/m²), obese older adults with BMI \geq 30 kg/m² experienced impairment in the activities of daily living approximately five years earlier and were twice as likely to develop functional impairments (Anton et al. 2013). The link between obesity and increased risk of frailty has been explained by the strong association between obesity and higher low grade inflammation (Soysal et al. 2016). In addition, obese individuals are less active and this may in the long run predispose to a loss of MM and strength.

In addition, age-related hormonal changes have been linked to frailty or to its components. Nonetheless, the few studies that have tried to explore the hormonal relationship between factors such as testosterone, growth hormone and IGF-1 and frailty, have been inconclusive (Bauer and Sieber 2008).

2.2.2 Frailty definition

Frailty can be defined as a state of augmented sensitivity and vulnerability to external stressors in old age and poor resolution of homeostasis after a stressor event, which increases the risk of adverse health outcomes, and disability (Clegg et al. 2013). It is known that frail people recover

slower after exposure to major or minor stressors when compared to their non-frail counterparts (Clegg et al. 2013). The syndrome of frailty has been hypothesized to be associated with increased vulnerability to stressors such as infection, injury, changes in medication that are encountered in many older adults (Rockwood et al. 2005). Thus, it is of the utmost importance to find a tool to measure frailty.

There are many operational definitions available for frailty, but there is no consensus definition which can be used in clinical practice. The operational definitions have been introduced to attempt to distinguish frail from non-frail older adults (Buta et al. 2016). The most commonly frailty instruments cited in the literature are physical frailty phenotype (Fried et al. 2001), deficit accumulation index (Rockwood et al. 2005), and the Gill frailty measure (Rothman et al. 2008). In 2001, Fried et al. (Fried et al. 2001) initially hypothesized some core clinical presentations of frailty in the Cardiovascular Health Study. The definition was operationalized utilizing data collected at baseline and at year 3; it has become the most commonly used definition of frailty. The participants were 5,317 men and women 65 years and older (4,735 from an original cohort recruited in 1989–90 and 582 from an African American cohort recruited in 1992–93). The Fried frailty phenotype classifies frailty as presence of three or more of the following five components: 1-Shrinking: unintentional weight loss (≥ 4.5 kg in prior year or, $\geq 5\%$ of body weight in prior year) by direct measurement of weight, 2- Weakness: grip strength in the lowest 20% at baseline, adjusted for gender and BMI, 3- Poor endurance and energy: as indicated by self-report of exhaustion. Self-reported exhaustion, identified by two questions from the Center for Epidemiologic Studies Depression Scale (CESD) (“I felt that anything I did was a big effort” and “I felt that I could not keep on doing things”) (Orme et al. 1986), 4- Slowness: The slowest 20% of the population was defined at baseline, based on time to walk 4.5 meters, adjusting for gender and standing height, 5- Low physical activity level: A weighted score of kilocalories expended per week was calculated at baseline: lowest 20% (males: 383 kcals/week and females: 270 kcals/week). The number of criteria (a 6-level ordinal variable ranging from 0 to 5) is

categorized into a 3-level variable depicting robustness (none of the criteria), pre-frailty (one or two criteria) and frailty (three or more criteria) (Cesari et al. 2014b, Fried et al. 2001).

Subsequently, Rockwood et al. (Rockwood et al. 2005) used the Canadian Study of Health and Aging to develop and validate the so-called frailty index. These workers created the frailty index using a checklist of a set of clinical conditions and diseases (Rockwood et al. 2005). They used 70 items in the original version which are not to be considered as a fixed set of variables. It has been reported that each 1-category increment of the scale significantly increased the medium-term risks of death and entry into an institution. The major drawback associated with the frailty index is that it requires a comprehensive geriatric assessment. However, once completed, the frailty index then becomes extremely informative for the continuous follow-up of the subject (Cesari et al. 2014b).

It might be inappropriate to view these two models as alternatives to each other as they are different and should rather be considered as complementary (Cesari et al. 2014b). According to Fried et al. , the frailty phenotype depicts a novel age-related condition that can exist even in the absence of clinical symptoms, whereas the Rockwood frailty index describes a profile closer to one measured by clinicians. The main characteristics and differences of these two definitions are presented in *Table 3*. The frailty phenotype can be applied at the first contact and does not need clinical evaluation.

Table 3. Main characteristics of the frailty phenotype and the Frailty Index

Frailty phenotype developed by Fried et al. (Fried et al. 2001, Rockwood et al. 2005)	Frailty Index developed by Rockwood et al. (Rockwood et al. 2005)
Signs, symptoms	Diseases, activities of daily living, results of a clinical evaluation
Possible before a clinical assessment	Doable only after a comprehensive clinical assessment
Categorical variable	Continuous variable
Pre-defined set of criteria	Unspecified set of criteria
Frailty as a pre-disability syndrome	Frailty as an accumulation of deficits
Meaningful results potentially restricted to non-disabled older persons	Meaningful results in every individual, independently of functional status or age

It is estimated that about a quarter of older individuals >85 years old are frail (Song et al. 2010). A recent systematic review carried out in 61,500 community-dwelling population aged 65 and older, estimated the overall prevalence of frailty to be 10.7%, whereas many more, 41.6%, were pre-frail (presence of one or two components of the Fried frailty index) (Collard et al. 2012). Nevertheless, because of the varied definitions of frailty status used in those studies, the reported prevalence differed substantially, ranging between 4.0–59.1 percent.

2.2.3 Sarcopenia and frailty

Sarcopenia and frailty have both received special attention in research, because both conditions are highly prevalent in the older people, associated with negative health-related events, potentially reversible, and relatively easy to evaluate in clinical practice. Sarcopenia and frailty are both highly relevant entities with regard to functionality and independence in the elderly (Bauer and Sieber 2008). Despite the relevance of sarcopenia and frailty for functionality and autonomy, there is still no consensus about their definitions. Thus, there has been a debate about whether sarcopenia is a component of frailty or whether these two phenomena should be considered as distinct geriatric conditions (Cesari et al. 2014a, Bauer and Sieber 2008).

To some extent, sarcopenia and frailty share common pathways; there is an overall agreement about the key role that physical function plays in the determination of the status of extreme vulnerability (Cesari et al. 2014a). Many experts have stated that exploring the potential causality between sarcopenia and frailty may be not sensible and therefore studying both in parallel is probably the best solution. “Determining whether frailty is due to sarcopenia, or sarcopenia is a clinical manifestation of frailty is consuming considerable efforts, and (from a very practical viewpoint) rather resembles the problem of “the egg and the chicken” (Cesari et al. 2014a). My aim in this literature review and doctoral thesis was to emphasize the medical and public health importance of both sarcopenia and frailty rather than evaluating their causality.

2.3 OSTEOPOROSIS AND SARCOPENIA

Osteoporosis is major public health problem, particularly in women (Simonen 1986); it represents a major non-communicable disease and its incidence will increase markedly in the future. Osteoporosis is mainly characterized by reduced bone mass and disruption of the bone structure, which consequently increases the risk of bone fragility and fracture risk. The societal and personal costs of osteoporosis are significant, i.e. it has been estimated that more than 75 million individuals in the United States, Europe and Japan are suffering from osteoporosis according to the criteria presented by WHO in 1994 (WHO 2004).

Bone mineral density (BMD) and bone mineral content (BMC) measured by DXA, have been considered as important determinants of osteoporotic fractures (Nguyen et al. 2005). Osteoporotic fractures are a major cause of morbidity in the older population. In particular, hip fractures causes severe pain and loss of independence and function, and most of the cases require hospitalization. Recovery after a fracture is slow, and rehabilitation is often incomplete, with many patients permanently institutionalized in nursing homes. Common sites of osteoporotic fractures are the spine, hip, distal forearm and proximal humerus. In the year 2000,

there were estimated to be 620,000 new fractures of the hip, 574,000 of the forearm, 250,000 of the proximal humerus and 620,000 clinical spine fractures in men and women aged 50 years or more in Europe. These fractures accounted for 34.8% of all fractures worldwide (Johnell and Kanis 2006). In Finland, there were 7,594 hip fractures recorded in 2010. It has been estimated that the number of hip fractures will increase by 1.8-fold by 2030 because the size of the 50-year-old or older population is likely to increase sharply in the near future (Korhonen et al. 2013). Currently, bone densitometry measurements are the most reliable methods to predict the future risk of fracture. However, the ability of such measurements to predict a future fracture is still matter of debate.

Bones and muscles develop and age together. It is not fully understood how bone senses mechanical loading of muscles, or which cells are responsible for this ability, and whether bone loses its mechanosensitive with aging (Karasik and Kiel 2010). The term “sarco-osteopenia” was coined for the first time in 2009 to emphasize that weak bones and weak muscles may contribute to fractures in older individuals. It has been suggested that the fracture risk could be attributed to the association between muscles and bones (Chalhoub et al. 2015). Muscles and bones share common genetic factors and are considered to be affected by pleiotropic genes which are responsible for the synchronized deterioration of both tissues with aging (Karasik and Kiel 2010).

Almost all previous studies in postmenopausal women have revealed that LM is correlated positively with whole-body and/or regional areal BMD (g/cm^2) (Bleicher et al. 2011, Ho-Pham et al. 2014). In addition, ALM was found to contribute significantly to regional BMD (Verschuere et al. 2013). A measure of muscle strength was found to be associated with BMD in postmenopausal women (Nguyen et al. 2000). In a Finnish study conducted by Rikkonen et al. (2012), women ($n=979$, and mean age 68.1 year) with osteoporosis had significantly smaller LMI, ALM, grip strength, and knee extension strength but not FM index (FM divided by height)

compared to their counterparts (Rikkonen et al. 2012). Grip and knee extension strength were 19 and 16 % weaker in osteoporotic women compared to their non-osteoporotic counterparts. In another study among 679 men (mean age 59.6 years), ALM, RSMI and FM were positively associated with BMD (Verschuere et al. 2013). Men with RSMI $<7.26 \text{ kg/m}^2$ had a significantly lower BMD value compared with those with RSMI $\geq 7.26 \text{ kg/m}^2$. Men with RSMI lower than EWGSOP cut off ($\leq 7.23 \text{ kg/m}^2$) were more likely to have osteoporosis compared with those with normal RSMI (Verschuere et al. 2013). It has also been suggested that sarcopenic (lowest tertile of ALM) and dynapenic ("age-associated loss of muscle strength that is not caused by neurologic or muscular diseases"), obese older individuals may have an increased risk of osteoporosis and non-vertebral fractures relative to obese, but not sarcopenic or dynapenic counterparts (Scott et al. 2016). The varied definition of sarcopenia in previous studies complicates the interpretation, however, current evidence suggests that there is a relationship between sarcopenia and bone in ageing.

A deterioration in muscle strength, MM and BMD may contribute to fractures and falls in the older population. Sarcopenia can lead to a higher risk of falls and functional impairments, which are considered as the common causes of fracture (Landi et al. 2012, Janssen et al. 2002). Moreover, myosteatosis, which is responsible for a loss of muscle strength and function, may be associated with fractures (Gielen et al. 2012). The combined effect of sarcopenia (defined as low MM and strength) and low BMD on fracture risk has been explored by Chalhoub et al. (2012), among men ($n=5,544$) and women ($n=1,114$) aged 65 and older (Chalhoub et al. 2015). Women with low BMD and sarcopenia ($\text{HR}=2.27$, 95% $\text{CI}=1.37\text{--}3.76$) and women with low BMD alone ($\text{HR}=2.62$, 95% $\text{CI}=1.74\text{--}3.95$), but not women with only sarcopenia, had a greater risk of fracture than women with normal BMD and no sarcopenia (Chalhoub et al. 2015).

Although only a limited number of epidemiologic studies have addressed the associations of sarcopenia and risk of falls, sarcopenia has been frequently mentioned as an important risk

factor for falls in older individuals. One study examined 796 men aged 50 to 85 years of age; the men in the highest tertile of relative RSMI ($>7.31 \text{ kg/m}^2$) were less likely to report a fall in the previous year compared with those in the lowest quartile of RSMI ($<6.32 \text{ kg/m}^2$) (Szulc et al. 2005). A common limitation of such studies was that the data about falls were collected retrospectively at the time of the MM assessment (Szulc et al. 2005, Baumgartner et al. 1998). Therefore, causality whether MM has been negatively affected by the experience of falls (e.g., caused by an increased fear of falling and related decreased physical activity level) or by the potential consequences of the fall (injuries) cannot be assessed. Furthermore, perhaps individuals cannot remember all of the times that they have fallen.

Other possible factor that may contribute to sarcopenia and osteoporosis is vascular disorders. Ageing is accompanied by several changes in the body inducing vascular ageing, which may intertwines with geriatric syndromes. In general, total body skeletal muscle contains main part of the small-vessel network in the body and also the major vascular resistance network (Strandberg et al. 2013). Thus, functional and effective blood flow is critical for muscle performance in the body, and small-vessel disease which hinder this may cause impaired blood flow and exacerbate sarcopenia by muscle atrophy (Lee et al. 2007). Osteoporosis has been linked to atherosclerosis, vascular calcification. It is known that appropriate blood circulation to the bone is required for bone constructions and function and therefore those with impaired vascular system have lowered BMD (Persyand D'Haese 2009).

2.4 ROLE OF NUTRITION IN MUSCULOSKELETAL HEALTH

Nutrition is regarded as an important factor contributing to the complex etiology of sarcopenia. Recently, several studies have assessed the role of nutritional factors and MM, muscle strength and physical function (Volkert 2011, Hickson 2015). Consequently, it has been speculated that modifying nutritional habits may also be able to prevent sarcopenia and frailty. Alternatively, sarcopenia and frailty may compromise adequate nutrition. Sarcopenic or frail individuals are

less active, and shopping and cooking may become burdensome for them. Thus, a vicious cycle may develop where sarcopenia and malnutrition mutually amplify one another (54).

The results of a study in a community-based study conducted by Bartali et al. (Bartali et al. 2006) in Northern Italy among more than 800 healthy subjects showed that an energy intake in the lowest quintile, i.e. below 21 kcal/kg BW and day, was related to physical frailty. Frailty was defined as having at least two of the following characteristics low muscle strength, feelings of exhaustion, low walking speed and reduced physical activity. After adjusting for energy intake, also a low intake of protein, vitamins D, E, C, folate and a combined effect of more than 3 nutrients at the same time, were significantly related to frailty. The review published by Schiaffino et al. (Schiaffino et al. 2013) comprehensively explored the role of various dietary factors to muscle synthesis and breakdown. In particular, protein and amino acids have been considered as the most important dietary factors in the prevention of sarcopenia. Vitamin D, antioxidants and polyunsaturated fatty acids may also contribute to the preservation of muscle function (Hickson 2015). Nevertheless, in this doctoral thesis, the main focus has been given to protein intake as the key nutrient in this context, since this is in line with our study's aims.

2.4.1 Recommendations of protein intake in the older individuals

The recommended dietary allowance (RDA) for protein intake for all men and women aged 19 years and older is 0.8 g/kg BW per day. This recommendation was established in 2005 by the Institute of Medicine and was based on short-duration nitrogen balance studies in young adults (Trumbo et al. 2002). Recently, concerns have arisen whether this amount is actually sufficient for older adults (0.8 g/kg BW). This amount of RDA protein intake (0.8 g/kg BW) may be insufficient to promote optimal health and preserve physical performance in the older individuals (Volpi et al. 2013, Lemieux et al. 2014). This has led to the recent appearance of dietary protein intake recommendations for the older individuals (Table 4). The PROT-AGE Study Group is an international study group, which recommended that the dietary protein intake

should be in the range of 1.0–1.2 g/kg BW in healthy older adults (Bauer et al. 2013). Furthermore, the 2012 Nordic nutrition recommendation (NNR) in particular suggested an optimal protein intake of up to at least 1.1–1.3 g protein/kg BW (approximately 15–20 energy %) for the older individuals. This would represent an increase of 20% compared to the NNR 2004 recommendation. Recent reviews and consensus statements have suggested that a dietary protein intake between 1.0 and 1.5 g/kg BW per day may provide health benefits beyond those afforded by simply meeting the minimum requirements (Volpi et al. 2013, Paddon-Jones et al. 2015).

Table 4 Dietary protein intake recommendation by different institutions for the elderly.

Recommended dietary allowance (Institute of Medicine of the National Academy of Sciences 2005)	0.8 g/kg body weight
PROT-AGE Study Group	1.0 –1.2 g/kg body weight – those with acute or chronic diseases: 1.2–1.5 g/kg BW
Nordic nutrition recommendation 2012	1.1–1.3 g protein g/kg BW

It has been estimated that about 10% of European community dwelling older adults and 35% of institutionalized adults fail to eat enough food according to estimated average requirement for daily protein intake (0.8 g/kg BW/day), which is a minimum intake to preserve musculoskeletal health in the adult population (Tieland et al. 2012a). The 2012 Findiet survey reported that the mean protein intake among the Finnish elderly women was 62 g/d (15 energy %) which is at the lower limit of the 2012 NNR recommendation (Helldán et al. 2013).

2.4.2 Role of protein intake in sarcopenia and frailty: selection of the studies

This is synopsis of the research regarding the effects of protein consumption on body composition (bone mass, and MM), physical function, sarcopenia and frailty in older adult populations. The following criteria were considered in conducting the literature review in this part:

- Participants aged 50 years and older in community-dwelling, hospital and nursing home/geriatric settings.
- The outcome measures reported for sarcopenia including MM and at least one measure of muscle strength or physical performance, even when the population studied was not defined as sarcopenic. Those studies which defined frailty according to Fried or Rockwood criteria or its surrogate were included. If these outcomes were not clearly stated within the study methodology, the study was excluded.
- For the protein intake as the main exposure, we included studies with protein intake from foods. The protein intake could be expressed as animal protein, plant protein and/or total protein (animal+ plant). Only a limited number of randomized control trials (RCTs) have examined the effect of protein-rich foods separately on sarcopenia and frailty, therefore, only intervention studies (and not observational studies) that used isolated protein as supplements and amino acids were included.

2.4.3 Dietary protein intake and sarcopenia

There are very few RCTs that have explored the effect of protein supplementation on sarcopenia. The results of these RCTs which have supplemented protein alone or combined with other nutrients or exercise are summarized in Table 5. In these studies, there was little evidence for benefits in functional status, and the measures were too heterogeneous to permit comparison. In only one study with no associated exercise intervention in 65 frail community-dwelling individuals, was the provision of 15 g milk protein per day at breakfast and lunch for 24 weeks able to improve physical performance, but not MM or muscle strength as compared to control group. In line with this, a systematic review by Cruz-Jentoft et al. (Cruz-Jentoft et al. 2014) did not find a consistent effect of protein supplementation on MM and function in sarcopenic elderly subjects.

Observational studies, although limited, have detected positive associations between protein intake with MM (Houston et al. 2008, Scott et al. 2010a, Meng et al. 2009a, Geirsdottir et al. 2013b), physical function, sarcopenia or frailty (Beasley et al. 2010) (Table 6). In a recent report from The Framingham Third Generation Study by Mangano et al. (2017) among subjects aged 40 ± 9 years, individuals in the lowest quartile <59 g/d (<0.8 g/kg BW) of total protein intake (g/d) had significantly lower ALM, ALM/height², and quadriceps strength than those in the higher quartiles of intake i.e. 129 g/d (1.8 g/kg BW) (Mangano et al. 2017). The findings of the study of Gregorio et al. (Gregorio et al. 2014) among 387 healthy women aged 60–90 years showed that those in the lower protein intake <0.8 g/kg BW category performed less well in the single-leg stance test than those in the higher protein intake ≥ 0.8 g/kg BW category. They also walked 8 feet at a slower pace, and their SPPB score was lower. Further, Lemieux et al. (Lemieux et al. 2014) indicated that among 72 postmenopausal women, a higher protein intake (≥ 1.2 g/kg BW) was positively correlated to hand grip strength and knee extension. The Women's Health Initiative clinical and longitudinal observational study (Beasley et al. 2013) followed-up 134,961 participants aged 50–79 years for an average of 7 years. The results showed that mean hand grip strength at baseline was slightly higher among women with a higher daily protein intake, and these women experienced a smaller decline in hand grip strength over time than those with low calibrated (calibrated against doubly labeled water and 24-hour urinary nitrogen) protein intake. In addition, women in the highest quintile of the calibrated protein intake on average completed more chair rises at baseline than women in the lowest quintile. In contrast, there was no significant association between calibrated protein intake and the timed 6-m walk in either cross-sectional or prospective analyses.

Several studies have explored the role of protein intake for preserving the MM in the older individuals. Houston et al. showed that among women aged 70–79 years ($n = 2066$), those with a higher protein intake (median: 19 % of energy intake, 60.7 g/d) lost 40 % less lean mass as compared with those with a lower intake (median: 11 % of total energy intake) over a 3-year

follow-up (Houston et al. 2008). Similarly, Meng et al. found that elderly women with a higher total protein intake (average >1.6 g/kg BW or 20.0 % of energy) had higher LM as compared with those with a lower protein intake (average 0.85 g/kg BW or 16.0 % of energy) (Meng et al. 2009b). Although, MM is an important predictor of sarcopenia and frailty, it does not display a linear correlation with physical function and muscle strength among older people.

There is some evidence that the anabolic response to amino acid intake may be blunted in older people, particularly if they have low intakes (Wolfe 2013). Current evidence suggests that ageing might result in an attenuation of muscle protein synthesis towards the anabolic effect of hyperaminoacidemia, particularly at lower protein intakes (Carbone et al. 2012, Shilland and McCarthy 2015, Paddon-Jones and Leidy 2014). Moore et al. (2015) claimed that the relative amount of protein required to maximize muscle protein synthesis was greater in older men as compared with their younger counterparts (Moore et al. 2015). Thus, basal muscle protein synthesis reached a plateau after ingestion of 0.40 ± 0.19 and 0.24 ± 0.06 g/kg LM and 0.60 ± 0.29 and 0.25 ± 0.13 g/kg LM in older and younger men, respectively. However, it is not established whether elderly individuals with greater LM have a higher capacity for muscle protein synthesis as compared to those with lower LM.

Table 5. Summary of the effect of protein, amino acids on sarcopenia and its indices in randomized, controlled studies.

Reference	Population; age; number (M/F)	Study setting	Outcomes measured	Main results
Rondanelli M et al. (2016)(Rondanelli et al. 2016)	Community-dwelling; 80.3 y; n=130 (53/77)	IG: WPS (22 g), EEA (10.9 g, including 4 g leucine), and vitamin D (100 IU) concurrent with regular, controlled PA CG: received none of above. Duration: 12 weeks.	MM (DXA), MS (GS)	SUP and exercise significantly increased MM, GS, PP in intervention group.
Bauer et al. (2015) (Bauer et al. 2015)	Sarcopenic elderly; ≥65 y; n=380 (133/247)	IG: The active group (n=184) received a vitamin D 800 IU, and leucine-enriched WPS 20 g to consume twice daily. CG: (n = 196) received an isocaloric control product. Duration: 13 weeks	MM (DXA), MS (SPPB, chair stand test, GS)	Vitamin D and leucine-enriched WPS resulted in improvements in MM and lower-extremity function.
Daly et al. (2014)(Daly et al. 2014)	Residents of 15 retirement villages; 60–90 y; n= 100 (0/100)	IG: received lean red meat (about 160 g cooked) to be consumed 6 days/week or control (1 serving pasta or rice/day). CG: All women undertook RET 2 times/week and received 1000 IU vitamin D/day. Duration: 4 months.	MM (DXA), MS (timed-up-and-go test, and 30 s sit-to-stand test, LE strength)	A protein-enriched diet equivalent to 1.3 g/kg/day achieved through lean red meat was significantly effective for enhancing the effects of progressive resistance training on LM and MS.

Table 5 continued

Reference	Population; age; number (M/F)	Study setting	Outcomes measured	Main results
Shahar et al. (2013)(Shahar et al. 2013)	Sarcopenic elderly; 60–74 y; n = 65 (47/18)	IG: (i) exercise group; (ii) soy protein drink (up to 1.5 g/kg/BW); or (iii) the combination of exercise and soy protein drink. CG: none. Duration: 12 weeks	MS (Senior Fitness Test)	The exercise program improved MS and body composition, protein SUP reduced body weight and increased upper body strength.
Aleman-Mateo et al. (2012)(Aleman-Mateo et al. 2012)	Patients with sarcopenia; ≥60 y; n = 40 (17/23)	IG: 210 g/day of ricotta cheese (15.7 g of protein) plus the habitual diet CG: habitual diet Duration: 3 months.	MM (DXA), MS (GS)	Adding ricotta cheese to the habitual diet improved the markers of sarcopenia without a pronounced loss of skeletal muscle mass and MS improved.
Kim et al. (2012)(Kim et al. 2012)	Sarcopenic women; ≥75 y; n = 155 (0/155)	Subjects were randomly assigned to RET and AA SUP (n = 38), exercise (n = 39), AA SUP (n = 39), or health education (n = 39). The exercise group attended a 60-min comprehensive training program twice a week, and the AA SUP group ingested 3 g of a leucine-rich EAA mixture twice a day for 3 months.	MM (BIA), MS (walking speed, KE)	Walking speed significantly increased in all three IGs, leg MM in the exercise + AA SUP and exercise groups, and KE only in the exercise + AA SUP group. The odds ratio for MM and KE improvement was more than four times as great in the exercise + AA SUP (odds ratio = 4.89, 95% confidence interval = 1.89–11.27) group than in the health education group.
Tieland et al. (2012)(Tieland et al. 2012b)	Frail elderly; n = 65 (not specified)	IG: SUP 15 g milk protein per day at breakfast and lunch Control group: placebo. Duration: 24 weeks.	MM (DXA), MS (leg press, LE, GS), PP (SPPB)	Dietary milk protein SUP improved PP, but did not increase MM.

Table 5 continued

Reference	Population; age; number (M/F)	Study setting	Outcomes measured	Main results
Dillon et al. (2009)(Dillon et al. 2009)	Healthy individuals; 68±2 y; n=14 (0/14)	IG: 15 g EAA CG: placebo Duration: 3 months.	MM (DXA), MS (bicep curl, triceps extension, LE, leg curl)	EAA improved LM and basal muscle protein synthesis.
Solerte et al. (2008)(Solerte et al. 2008)	Sarcopenic elderly subjects; 66–84 y; n = 41 (not specified)	IG: 8 g of EAA snacks twice a day CG: placebo. Duration: 16 months.	MM (DXA) MS (SPPB, maximal LE)	Significant increases in whole-body LM in all areas were seen.
Flakoll et al. (2004)(Flakoll et al. 2004)	Community-dwelling; 76.7 y; n= 57 (0/57)	IG: 2 g HMB, 5 g arginine, and 1.5 g lysine daily CG: placebo. Duration: 12 weeks.	MM (BIA), MS (isometric LE, GS, get up and go)	MS and PP significantly improved with arginine + HMB + lysine versus placebo
Bonnefoy et al. (2003)(Bonnefoy et al. 2003)	Frail, care institution; 83 y; n= 57 (7/50)	IG: 15 g of proteins, 25 g of carbohydrates, and 4.4 g of lipids. CG: placebo Duration: 9 months.	MM (labelled water), MP, PP (chair rise, 6-min walk, stair climb)	SUP significantly increased MS at 3 months versus control group, but not at 9 months

BIA, bioelectrical impedance analysis; CT, computerized tomography; DXA, dual-energy X-ray absorptiometry; EAA, essential amino acid; F, female; FFM, fat free mass; HMB, β-hydroxy β-methylbutyrate; GS, grip strength; KE, knee extension; LE, leg extension; M, male; MM, muscle mass; MS, muscle strength; PP, physical performance; RET, resistance exercise training; SPPB, short physical performance battery; SUP, nutritional supplement; VAL, valine; WPS, whey protein supplement.

Table 6. Summary of the association of protein intake with sarcopenia and its indices in observational studies.

Reference	Population ; age; number (M/F)	FU	Food record method/protein intake categories	Outcomes measured (method)	Covariates adjustment in the final analytical model	Main results
Mangano et al. (2017)(Mangano et al. 2017)	Framingham Third Generation Study; 19–72 y; n=2986 (M/F not specified)	3 y	FFQ/dietary protein food groups	aLM; MS (QS)	sex, estrogen status, age, BMI, height, EI, smoking, supplemental calcium, supplemental vitamin D, and PA	Individuals in the lowest quartile of TP intake (quartile 1) had lower aLM, aLM/ht ² , and QS than those in the higher quartiles of intake.
Sahni et al. (2015)(Sahni et al. 2015)	Framingham Offspring Cohort; 59 y; n=2675 (1166/1509)	CS	FFQ/quartiles of energy-adjusted TP, AP and PP	LM, Leg LM (DXA); MS (QS)	age, height, EI, PA, health status, and women's menopause status	In men and women, leg LM was higher in the highest quartiles of TP and AP intake compared with those in the lowest quartiles.
Lemieux et al. (2014)(Lemieux et al. 2014)	62 y; n= 72 (M/F not specified)	CS	3-day food record/two groups protein intake ≥1.2 g/kg BW and 0.8 – 1.19 g/kg BW	Body composition (DXA); MS (GS and KE)	MM, FM, essential amino acid intake and non-essential amino acid intake	Women with protein intake ≥1.2 g/kg BW had a higher MS and lower BMI and FM compared to women with protein 0.8 – 1.19 g/kg BW.

Table 6 continued

Reference	Population; age; number (M/F)	FU	Food record method/protein in intake categories	Outcomes measured (method)	Covariates adjustment in the final analytical model	Main results
Gregorio et al. (2014)(Gregorio et al. 2014)	Healthy women; 72±7 y; n= 387 (0/387)	CS	4-day food record/protein intake upper or lower 0.8 g/kg BW.	LM, aLM, and FM (DXA); PF (PPT, SPPB); MS (GS)	BMI	Women in the higher protein group had lower body mass, including FM, LM, fat-to-lean ratio, lower PPT and SPPB than those in the lower-protein group.
Geirsdottir et al. (2013)(Geirsdottir et al. 2013b)	Older adults; 50–79 y; n= 237 (M/F not specified)	CS	3-day food record/protein intake (g/kg BW) quartiles	MM (DXA); PF (time up and go, 6 min walk)	age, number of drugs, PA	TP (g/kg BW) was associated with LM. The differences in LM were 2.3 kg and 2.0 kg between the fourth vs the first and the fourth vs the second quartiles, of TP, respectively.
Beasley et al. (2013)(Beasley et al. 2013)	Women's Health Initiative; 50–79 y; n=134,691 (0/134691)	7 y	FFQ/quartiles (g/d, g/kg BW)	Self-reported PF (short-form RAND-36), GS, number of chair stands in 15 seconds, and timed 6-m walk	age, income, education, race and ethnicity, BMI, smoking, alcohol consumption, PA, hormone therapy use, living alone, number of falls, disability, depression, self-reported history of medical conditions.	Higher calibrated TP was associated with higher self-reported PF and slower rate of functional decline.

Table 6 continued

Reference	Population; age; number (M/F)	FU	Food record method/protein in intake categories	Outcomes measured (method)	Covariates adjustment in the final analytical model	Reference
Morris et al. (2013)(Morris and Jacques 2013)	free-living people; ≥ 50 y; n = 2425 (M/F not specified)	CS	Two 24-h diet recalls/protein intakes per kg BW < 0.8, 0.8-1.0 g and >1.0. And continuous variable was per 10g increase.	aLM (anthropometric measures)	Age, sex, race-ethnicity, smoking status and physical activity.	High-protein intake was associated with a modest increase in the appendicular skeletal muscle mass index in non-obese, physically inactive subjects.
Bartali et al. (2012)(Bartali et al. 2012a)	The Invecchiare in Chianti Study; n=598 (282/316)	3 y	FFQ/as a continuous variable	MS (KE)	Age, sex, BMI, EI, chronic conditions, physical activity, smoking, and muscle strength at baseline.	The main effect of protein intake on change in muscle strength was not significant.
Scott et al. (2010)(Scott et al. 2010b)	community-dwelling older adults; 75 ± 3 y; n=740 (not specified)	2.6 y	FFQ/as a continuous variable	aLM (DXA)	Age, sex, aLM at baseline, and change in physical activity and body fat.	TP was associated with lower aLM at baseline (-0.81 kg) and follow-up (-0.79 kg).

Table 6 continued

Reference	Population; age; number (M/F)	FU	Food record method/protein in intake categories	Outcomes measured (method)	Covariates adjustment in the final analytical model	Reference
Meng et al. (2009)(Meng et al. 2009a)	elderly postmenopausal women; 75±3 y; n = 862 (0/862)	5 y	FFQ/tertiles of TP (g/d)	LM, UAMA (DXA)	age, height, percentage leg fat, EI, physical activity, health status, and women's menopause status	Compared with those in the lowest tertile of protein intake (<66 g/d), women in the top tertile (>87 g/d) had 5.4–6.0% higher whole body and appendicular lean mass and UAMA.
Houston et al. (2008)(Houston et al. 2008)	Health ABC study; 70–79 y; n = 2066 (M/F not specified)	3 y	108-item FFQ/ quintiles of energy-adjusted TP	Changes in LM and aLM (DXA)	age, sex, race, study site, total EI, baseline aLM, height, smoking, alcohol use, PA, oral steroid use, prevalent disease, and interim hospitalizations.	Participants in the highest quintile of protein intake lost ≈40% less LM and aLM than those in the lowest quintile of protein intake.
Lord et al. (2007)(Lord et al. 2007)	Older women; 66±5 y; n=38 (0/38)	CS	3-day food record/TP, AP and PP (g/d)	MM index (DXA)	None	AP was associated with MM index.

BMI, body mass index; BW, body weight; FFQ, food-frequency questionnaire; FM, fat mass; FU, follow up; M, male; F, female; GS, grip strength; LM, lean mass; aLM, appendicular lean mass; y, year; EI, energy intake; PA, physical activity; MS, muscle strength; QS, quadriceps strength; TP, total protein; AP, animal protein; PP, plant protein; CS, cross-sectional; UAMA, upper arm muscle area, KE, knee extension; PF, physical function; SPPB, Short Physical Performance Battery; PPT, physical performance test

2.4.4 Dietary protein intake and frailty

As far as we are aware, the study of Tieland et al. (2012) (Table 6) is the only RCT, which has assessed the impact of 24 weeks of dietary protein supplementation on MM, muscle strength, and physical performance in frail elderly people (n=65) (Tieland et al. 2012b). Subjects received either daily protein or placebo supplementation (15 g protein at breakfast and lunch). It was reported that dietary protein supplementation improved physical performance, but did not increase skeletal MM in frail elderly people.

There are few observational studies on the association of protein intake and frailty, their results are summarized in Table 7. There seems to be only one large longitudinal cohort study; Beasley et al. (2013) analysed the data of the Women's Health Initiative study, among 24417 women aged 65 to 79. They reported that a 20% greater protein intake (% of energy) (the mean protein intake was 1.2 g/kg BW) was associated with a 9% lower risk of prefrailty and a 12 % lower risk of frailty (Beasley et al. 2010). The results of the French Three-City cohort examining 1345 community dwelling-older adults aged 65 years and older, were that a protein intake ≥ 1 g/kg BW was associated with lower prevalence of frailty (defined by Fried criteria), and slowness (indicated by low walking speed) (Rahi et al. 2016). Further, among 2108 grandmothers or acquaintances of nutrition students aged 65 years and older in Japan, total protein intake was significantly inversely associated with frailty. In a cross-sectional study, those subjects who were categorized to the third, fourth, and fifth quintiles of total protein intake (>69.8 g/d) showed significantly lower ORs of frailty than those in the first quintile (Kobayashi et al. 2013). Bollwein et al. examined 194 community-dwelling seniors aged 75 years and older; frail participants consumed less protein in the morning, and more at noon; however, there were no significant differences in the risk of frailty when the higher protein quartile intake was compared to the lowest quartile (Bollwein et al. 2013).

Table 7. Summary of the association of protein intake with frailty in observational studies.

Reference	Population; age; number (M/F)	FU	Food record method/protein intake categories	Frailty ascertainment	Covariates adjustment in the final analytical model	Main results
Rahi et al. (2016)(Rahi et al. 2016)	Bordeaux sample of the Three-City study- community-dwelling older adults; ≥65y; n=1345 (M/F not specified)	CS	24-hour dietary recall during /≥1 g/kg BW, and optimal energy intake ≥30 kcal/kg.	≥3/5 Fried criteria: weight loss, exhaustion, muscle weakness, slowness, and physical activity.	BMI, diabetes, cardiovascular history, depression, cognitive performance, and number of drugs. Model 2 for protein intake was additionally adjusted for total energy intake.	In the study sample, 55 (4.1%) were identified as frail. Higher protein intake was significantly associated with a lower frailty prevalence, whereas no significant association was observed between an optimal energy intake and the presence of frailty.
Kobayashi et al. (2013)(Kobayashi et al. 2013)	Japanese community-dwelling older women; ≥65 y; n=2108 (0/20108)	CS	self-administered diet history/quartile s g/d	≥3/4 of the following components: slowness and weakness (two points), exhaustion, low physical activity, and unintentional weight loss.	Age, BMI, residential block, size of residential area, living alone, current smoking, alcohol drinking, and dietary supplement use, history of chronic disease, depression symptoms, and energy intake.	The number of subjects with frailty was 481 (23%). Total protein intake >69.8 g/d was significantly associated with lower ORs of frailty. The intakes of animal and plant protein and all selected amino acids were also inversely associated with risk of frailty.

Table 7 continued

Reference	Population; age; number (M/F)	FU	Food record method/protein in intake categories	Frailty ascertainment	Covariates adjustment in the final analytical model	Main results
Bollwein et al. (2013)(Bollwein et al. 2013)	community-dwelling seniors; ≥75 y; n=194 (68/127)	CS	FFQ/quartiles (% energy and g/kg BW)	≥3 of the following criteria: weight loss, exhaustion, low physical activity, low handgrip strength and slow walking speed.	Age and sex, IADL score, number of medications, and chewing difficulties.	15.4% of the participants were frail, 40.5% were pre-frail. The risk of frailty did not differ significantly between participants in the higher compared to the lowest quartile of protein intake.
Beasley et al. (2010)(Beasley et al. 2010)	Women's Health Initiative Observational Study; 65–79 y; n=24417 (0/24417)	3 y	FFQ/a 20% increase in protein intake (% kcal) and g/kg BW.	≥3 of the following criteria: low physical function (measured using the Rand-36 questionnaire), exhaustion, low physical activity, and unintended weight loss.	Income, education, healthcare provider, smoking, alcohol, health status, comorbid conditions, hormone therapy use, number of falls, living alone, disabled, depressive symptoms, and log-transformed calibrated energy intake.	13.5% developed frailty, 29.8% prefrailty over 3 years. A 20% increase in uncalibrated protein intake (%kcal) was associated with a 12% lower risk of frailty.

BMI, body mass index; BW, body weight; FFQ, food-frequency questionnaire; M, male; F, female; y, year; CS, cross-sectional;

2.4.5 Dietary protein intake and bone health

A considerable number of studies have recently focused on the role of dietary protein in bone health, exploring modifiable ways to maintain bone health in the elderly. Adequate dietary protein intake seems to be important for skeletal health, as protein constitutes about half of the bone volume and a third of its mass. Calcium is the predominant mineral in the bone matrix, but protein is also a major part within that matrix. Moreover, protein might increase the protein-sensitive anabolic mediators related to calcium such as IGF-1 and increase intestinal calcium absorption (Thorpe and Evans 2011). The influence of protein on bone health might also be dependent on the presence of other factors. It is still unknown whether the relationship between protein intake and bone health is modified by calcium intake. Since dietary calcium can act as a buffer, a greater dietary acid load caused by protein, might be offset by adequate calcium intake. Some studies have explored the role of protein intake for bone health from the viewpoint of fracture risk and BMD, this doctoral thesis will focus on the protein intake role in BMD and BMC.

Rather few studies have examined the association between protein intake and bone health outcomes. In a meta-analysis of cross-sectional studies of protein intakes and BMD conducted by Darling et al. (2009), no association or only a small positive association was found (Darling et al. 2009). Sahni et al. (Sahni et al. 2014) showed that protein intake was positively associated with femoral neck (FN), trochanter and lumbar spine (LS) BMD in women, but no significant associations were seen in men at any bone site. In contrast, Darling et al. (Darling et al. 2011) examined 176 postmenopausal women (aged 58 years and older) and reported that protein intake was negatively associated with LS and FN BMD as well as FN BMC. In the more recent meta-analysis of Shams-White et al. (Shams-White et al. 2017), it was suggested that higher protein intake (>90 g protein/d, 25% and 30% of total energy, and 1.4 g g/kg BW) may exert a

protective effect on LS BMD as compared with lower protein intake (<80 g protein/d, 15% and 18% of total energy, and 0.8 g/kg BW) but that it had no effect on total hip, FN, or total body BMD or bone biomarkers. They found no support for combining protein calcium and vitamin D on LS BMD, total hip BMD, or forearm fractures; there was insufficient evidence for FN BMD and overall fractures. However, the authors acknowledged that previous studies were heterogeneous and confounding could not be excluded. Further studies are needed to clarify role of dietary protein in bone health.

2.4.6 Possible role of other dietary factors in sarcopenia and frailty

I have reviewed the published articles about the effects of nutritional factors on sarcopenia. Although the possible effect of other nutrients may be important, the following dietary factors and nutrients have received the greatest attention in RCTs and observational studies.

Energy intake

The basal metabolic rate and energy requirements change as a result of aging, partly due to the reduced amount of MM. Intake of food and energy in general decrease due to physiological changes such as reduced appetite, altered taste and smell sensations, or because of physical and mental impairments, chewing or swallowing problems (Brownie 2006, Nieuwenhuizen et al. 2010b). Chronic inadequate energy intake can result in muscle atrophy and may compromise mitochondrial energy metabolism in muscle fibres and lead to muscle fatigue, weakness and debility (Drummond et al. 2009). In older adults, a reduced energy intake has been reported to result in weight loss, and intentional or unintentional weight loss affects MM more than in younger adults (Baumgartner 2000, Rolland et al. 2008). Furthermore, regaining LM is more difficult in older than in younger people. The Health, Aging and Body Composition Study evaluated a large cohort study of elderly men and women aged 70–79 years. It was shown over a four-year period that during weight loss, significantly more LM was lost than was gained

with weight gain. Declines in MM have been claimed to predict a reduction in muscle force and performance (Reinders et al. 2017).

Vitamin D

A recent meta-analysis of RCTs suggested that vitamin D supplementation had no significant effect on MM (Beaudart et al. 2014a). A small number of studies have previously evaluated the relationship between low serum 25-hydroxyvitamin D levels and frailty, but these studies have been in disease-limited patient populations or have employed nonstandard definitions of frailty. Wilhelm-Leen et al. (2010) found that low serum 25-hydroxyvitamin D concentrations were associated with frailty amongst older adults ($n = 5048$, >60 years old) (Wilhelm-Leen et al. 2010). The results of vitamin D supplementation in sarcopenic or frail subjects have yielded inconclusive findings (Robinson et al. 2012). As far as is known, there is no evidence from any intervention reporting a positive effect of vitamin D supplementation on MM or physical function in the older individuals (Rejnmark 2011). More observational and interventional studies are needed to clarify the exact role of vitamin D in the pathophysiology and treatment of sarcopenia.

Antioxidants

Little is known of the association between antioxidants and sarcopenia and frailty. The mechanism underlying this potential association might be that oxidative stress and chronic inflammation are regarded to play important roles in age-related muscle atrophy and functional decline. The overproduction of reactive oxygen species evokes molecular damage in human skeletal muscle. Reactive oxygen species are also modulators of the production of proinflammatory cytokines such as $\text{TNF-}\alpha$, and IL-6. In the Women's Health and Aging Study in older community-living women, oxidative protein damage was found to be related to lower grip strength (Howard et al. 2007). Antioxidants are found in many plant-derived foods, mainly

in vegetables and fruits. Among older men and women in the InCHIANTI study, higher plasma carotenoid concentrations were associated with a lower risk of developing a severe walking disability over a follow-up period of 6 years (Alipanah et al. 2009).

N-3 polyunsaturated fatty acids

The results of a recent study in 44 healthy men and women aged 60–85 years have shown that consumption of fish oil–derived n-3 polyunsaturated fatty acids increased thigh MM, handgrip strength, and muscle strength measures compared with the control group (Meng et al. 2009b). The exact mechanisms by which fish oil–derived n-3 polyunsaturated fatty acids increase MM are not known but are thought to involve alterations in both anabolic and catabolic pathways. It has been suggested that polyunsaturated fatty acids may increase the rate of muscle protein synthesis (Gingras et al. 2007), and possibly exert a beneficial effect on the muscle lipid content and mitochondrial function, which are important determinants of muscle function.

2.4.7 Dietary patterns and sarcopenia, sarcopenia indices and frailty

The literature on dietary pattern analysis are very heterogeneous and extensive, and a detailed review of these studies is beyond the scope of this doctoral thesis. Although dietary patterns may have important roles in bone health, the literature review was narrowed to the role dietary patterns on sarcopenia, sarcopenia indices and frailty.

Recently, dietary pattern analysis has received considerable attention as an alternative approach to examining the association of a whole diet and the risk of chronic disease and health status. Most of studies that have assessed the role of nutrition in the older individuals have focused on specific components of foods, often single nutrients. However, the role of a single nutrition factor is often small and difficult to capture in observational studies; also people eat food, not nutrients (Mathers 2015). In addition, there is growing interest in using dietary quality indices to evaluate whether concordance to a certain dietary pattern or current dietary

guidelines lowers the risk of some disease. A dietary score represents a summary value of consumed foods or nutrients and characterizes a measure of concordance to a predefined (healthy) diet. Diet quality is in general measured by a higher intake of beneficial foods (such as whole grains, vegetables, fruits, and fish).

Several dietary patterns have been developed according to the dietary recommendation and food culture of different populations. The most commonly used dietary patterns which have been evaluated for their potential association with sarcopenia and physical function decline include healthy eating index (HEI), Mediterranean diet (MED), and Baltic Sea diet (BSD) (Kanerva et al. 2013, Trichopoulou and Vasilopoulou 2000b). However, very few studies have explored the association of dietary patterns with sarcopenia and frailty.

HEI was developed based on a 10-component system of five food groups, four nutrients, and a measure of variety in food intake (T Kennedy Eileen et al. 1995). Each of the 10 components has a score ranging from 0 to 10, so the total possible index score is 100. Components 1 through 5 measure the degree to which a person's diet conforms to the serving recommendations of the USDA Food Guide Pyramid for five major food groups: grains, vegetables, fruits, milk, and meat. Other components were overall fat consumption as a percentage of total food energy intake, saturated fat consumption as a percentage of total food energy intake, cholesterol intake, sodium intake, and the amount of variety in a person's diet (T Kennedy Eileen et al. 1995). No studies on the association of HEI with risk of sarcopenia or frailty in the older individuals were found. One study examined older adults aged 60 years or older in the 1999–2002 National Health and Nutrition Examination Survey; total HEI-2005 scores were positively associated with both gait speed and knee extensor power (Xu et al. 2012).

MED has been the most frequently used dietary pattern which has been studied for its role in chronic disease and also sarcopenia and physical function in the older individuals. Although

the definition of MED is heterogeneous and different cut-off values for consumption of food groups and quantification of each food components have been reported, the main characteristics of the MED diet were created by Trichopoulou et al. in 1995. Their definition was based on the sex-based median amount of consumption of food groups of the traditional Mediterranean diet in the sample that they investigated (Trichopoulou and Vasilopoulou 2000a). The main MED food groups are divided to higher concordant components including fruits, vegetables, legumes, cereals, fish, and olive oil and lower concordant components including meat and meat products, dairy products and alcohol (Sofi et al. 2014).

The results of the InCHIANTI study (Milaneschi et al. 2011) indicated that higher concordance to MED was associated with better lower body performance. Participants with higher concordance experienced a lesser decline in SPPB score, at the 3, 6 and 9 year follow-up, compared to those with lower concordance. Shahar et al. (Shahar et al. 2012) studied 2225 well-functioning men and women aged ≥ 70 years with over 8 years of follow-up; both usual and rapid 20 m walking speed declined in the three MED concordance groups; however, the group with the highest concordance to the MED performed better at all-time points. Further, after a 6-y follow-up, a higher concordance to MED was associated with lower odds of developing frailty (defined by Fried index) compared with those with lower concordance. Among 690 community-living persons (≥ 65 years), a higher concordance to a MED at baseline was also associated with a lower risk of low physical activity and low walking speed but not with feelings of exhaustion and poor muscle strength (Milaneschi et al. 2011).

The SYSDIET study of the University of Eastern Finland, conducted in conjunction with the Finnish Heart Association and the Finnish Diabetes Association, released a BSD Pyramid in January 2011 in order to illustrate the healthier choices of the diet consumed in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) (Uusitupa and Schwab 2011). Many foods cultivated in the Nordic countries, for example, apples and berries, rye, rapeseed oil,

salmon and dairy products, are considered to have health-enhancing features. It has been shown that BSD may be associated with a lower risk of abdominal obesity (Kanerva et al. 2013, Kanerva et al. 2014) and depressive symptoms (Ruusunen et al. 2013). As far as is known, in only one recent prospective study among older individuals women and men (mean age of 61 years) was a higher concordance to a healthy Nordic diet (similar to BSD) associated with better physical performance 10 years later, such as in the 6-min walk, arm curl and chair stand tests, reflecting better aerobic endurance and upper- and lower-body strength (Perala et al. 2016).

3 *Aims of the study*

The aim of this doctoral thesis was to investigate protein intake and dietary patterns and their associations with sarcopenia and frailty in a population sample of Finnish elderly women aged 65–72 years.

The specific aims of the doctoral thesis were:

1. To assess the association of intakes of total protein, animal protein and plant protein with BMD and BMC at LS, FN and total body (*Study I*),
2. The primary objective was to assess the associations of intakes of total protein, animal protein and plant protein with MM at baseline and with changes during a 3-year follow-up. A secondary objective was to evaluate the association of total protein intake with any change of LM according to weight-change status (*Study II*),
3. To examine the differences in muscle strength and physical function in elderly women with higher protein intake in comparison to those with a lower intake at the baseline and during a 3-year follow-up. Another aim was to examine the associations of total body FM and MM with physical function and muscle strength measures (*Study III*),
4. To evaluate the association of dietary protein intake, overweight and obesity with frailty (*Study IV*).
5. To investigate the association of a healthy diet as measured by the Baltic Sea diet and Mediterranean diet patterns with indices of sarcopenia (*Study V*),

4 *Methods*

4.1 **Study design and study population**

The Osteoporosis Risk Factor and Prevention (OSTPRE) Study is an ongoing observational study launched in Kuopio region, Finland in 1989 with the most recent follow-up being performed in 2014 (Kärkkäinen et al. 2010). This doctoral thesis used the data from the Osteoporosis Risk Factor and Fracture Prevention Study (OSTPRE-FPS). The primary aim of the OSTPRE-FPS study was to determine whether vitamin D (800 IU/d) and calcium supplementation (1000 mg/d) are effective in preventing falls and fractures in postmenopausal women. The inclusion criteria were age 65 years or older, still living in the region, willing to participate, and non-participation in any former trials or bone densitometry measurements of the OSTPRE Study. A detailed participant flow diagram is presented in Figure 3. This study was a secondary analysis on the subjects from the OSTPRE-FPS study. The power analysis was done based on the incidence of fractures. There was no a priori power analysis to calculate the size of the subsample of 750 women randomly selected from the 3,432 women at the baseline. All clinical measurements were performed in Kuopio Musculoskeletal Research Unit of the Clinical research center of the University of Kuopio. All participants provided written permission for participation. The study was approved in October 2001 by the ethical committee of Kuopio University Hospital. The study is registered in Clinical trials.gov by the identification NCT00592917.

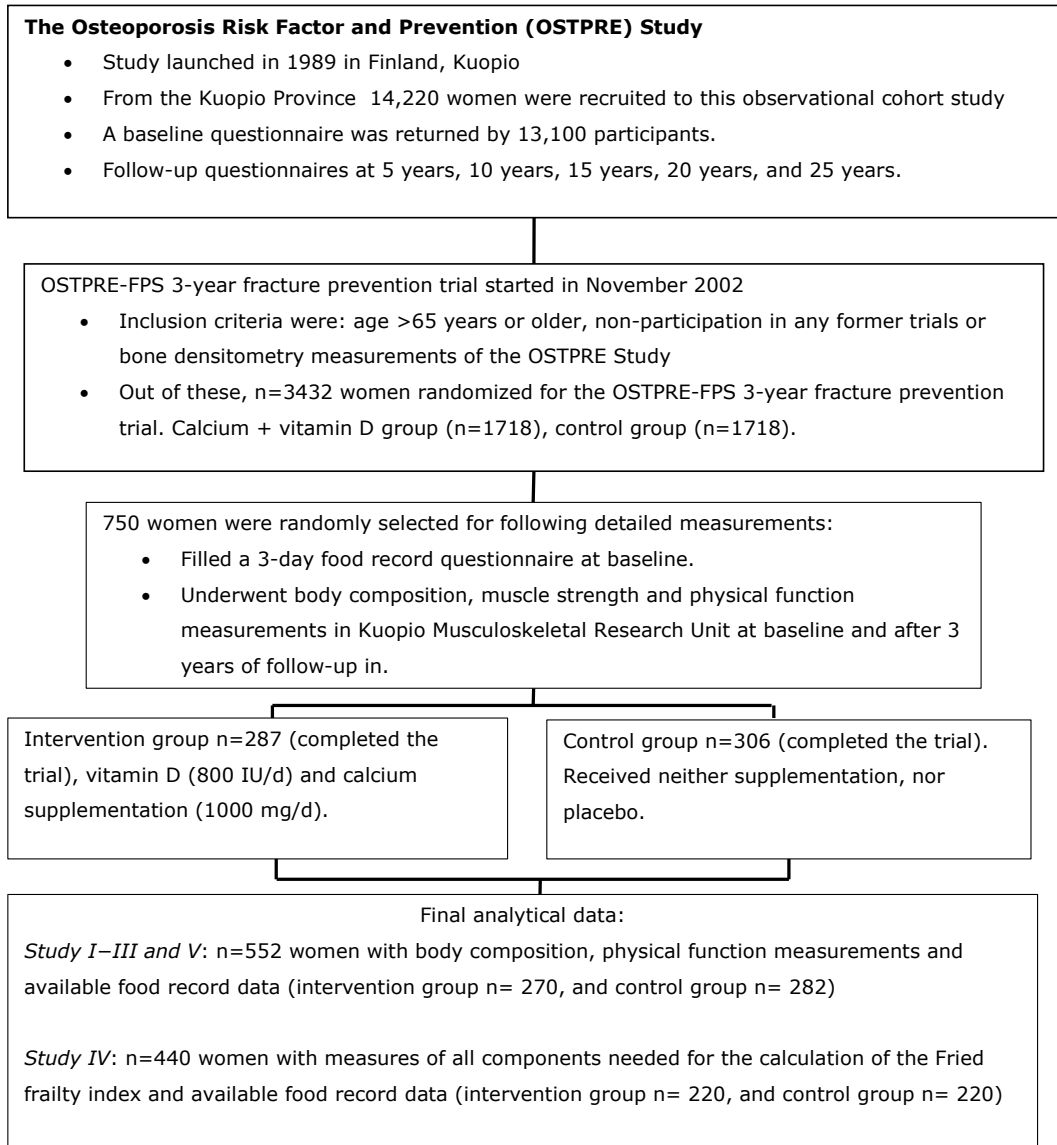


Figure 3. Osteoporosis Risk Factor and Fracture Prevention Study (OSTPRE-FPS) participant flow diagram

4.2 Dietary intakes

Data on food consumption was collected by using 3-day food record at baseline. A questionnaire and instructions were sent to the participants beforehand, and these were returned on the day that they visited the research site. Participants were advised to fill in the questionnaire for 3 consecutive days, including 2 days during the week and one day at the weekend (Saturday or Sunday). Participants were instructed to write down everything they ate and drank and to evaluate the amount of food consumed using household measures. In the case of uncertainties in the food record, a nutritionist called the participant for additional information (Erkkilä et al. 2012). Consumption of foods and the intake of nutrients were calculated using the Nutrica program (version 2.5, Finnish Social Insurance Institute, Turku, Finland). To assess if there was underreporting, the ratio of energy intake to estimated basal metabolic rate was calculated based on BW according to equations issued by Department of Health in the United Kingdom (Department of Health 1991). The ratio of energy intake to the basal metabolic rate cutoff value for under-reporting was chosen to be 1.49, as derived from Goldberg et al. (1991) (Goldberg et al. 1991) and Black (2000)(Black 2000) and none of the participants was excluded from the analyses (Isanejad et al. 2016).

Baltic Sea diet score

We devised a BSD score with slight modifications due to different dietary assessment methods in earlier studies (Kanerva et al. 2014). The final BSD score consisted of nine components, of which five were foods or food groups and four nutrient intakes. The BSD score components included 1) fruits and berries, 2) vegetables (root vegetables, legumes, nuts, mushrooms and vegetable products– potatoes excluded), 3) fiber from cereal products, 4) low-fat milk (skim milk and milk with fat content less than 2 %), and 5) total fish intake as positive components and 6) sausage as a negative component. 7) Total fat intake was expressed as a % of total energy

intake (% of energy). 8) Quality of fat intake was represented by calculating a ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acid (SFA). 9) Frequency of consumption of alcohol portions during past 4 weeks (1 portion=12 g) was asked in a separate questionnaire. The score construction is presented in Table 8. BSD score ranged from 0–25, higher points indicating higher concordance to BSD.

Mediterranean diet score

A predefined MED score was selected based on the existing literature, particularly on those studies that have applied the MED score in Nordic cohorts (Tognon et al. 2011, Bamia et al. 2013), as well as on the suggested positive association of MED score with physical function (Shahar et al. 2012). The score comprised of six positive components, including 1) high intake of root vegetables, legumes and nuts, mushrooms and vegetable products (potato excluded), 2) high intake of fruit, 3) high intake of cereals and potatoes, 4) high intake of fish, 5) high PUFA+monounsaturated fatty acid (MUFA): SFA ratio (as surrogate of quality of dietary fat), and 6) moderate alcohol intake. Two negative components were included 7) total meat including sausage and eggs, and 8) total milk and dairy products. The construction of the MED score is described in Table 8. Those who met all of the MED score criteria received a score of 8, reflecting maximum concordance.

Table 8. Construction of the Baltic Sea diet and Mediterranean diet scores.

Baltic Sea diet score components	
Components	Scoring
Total fruit and berries (g/d)	Q1=0, Q2=1, Q3=2, Q4=3
Vegetables: root vegetables, legumes and nuts, mushrooms and vegetable products (potato excluded) (g/d)	Q1=0, Q2=1, Q3=2, Q4=3
Fiber from total cereal products (g/d)	Q1=0, Q2=1, Q3=2, Q4=3
Fish (g/d)	Q1=0, Q2=1, Q3=2, Q4=3
Milk, low fat <2% (g/d)	Q1=0, Q2=1, Q3=2, Q4=3
Processed meat products, sausage (g/d)	Q1=3, Q2=2, Q3=1, Q4=0
Ratio of PUFA: SFA	Q1=0, Q2=1, Q3=2, Q4=3
Total fat intake (% of energy)	Q1=3, Q2=2, Q3=1, Q4=0
Alcohol (g/d) ^a	≤ 12 g/d=1 and otherwise=0
Mediterranean diet score components	
Components	Scoring
Vegetables: root vegetables, legumes and nuts, mushrooms and vegetable products (g/d)	≥ Median intake =1, < Median intake=0
Total fruits (g/d)	≥ Median intake =1, < Median intake=0
Total cereals and potatoes (g/d)	≥ Median intake =1, < Median intake=0
Fish (g/d)	≥ Median intake =1, < Median intake=0
Ratio of PUFA+MUFA: SFA	≥ Median intake =1, < Median intake=0
Total meat including sausage, and eggs (g/d)	≥ Median intake =0, < Median intake=1
Total milk and dairy products (g/d)	≥ Median intake =0, < Median intake=1
Alcohol (g/d) ^a	5 to 25 g/d =1 and <5 and >25 g/d=0

Q, quartile; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.

^a One portion of alcohol was calculated as 12 g.

4.3 Potential confounders

All lifestyle related information was gathered from the self-administered questionnaire. The OSTPRE-FPS questionnaire at the baseline assessed the information data on age at menopause (years), time since menopause (year), hormone therapy use (never used or used), duration of hormone therapy (years), smoking status (never, former and current), self-reported calcium

and vitamin D supplementation, use of alcohol (g/week); the use of medications possibly affecting bone metabolism including corticosteroids, diuretics, cytotoxic drugs, anticonvulsive drugs, anabolic steroids, calcitonin, bisphosphonates, and vitamin D. The assessed diseases possibly affecting BMD included hyperthyroidism, disease of the parathyroid gland, chronic liver diseases, chronic intestinal disease, celiac disease, ventricle operation, chronic nephropathy arthritis, osteoporosis, and lactose intolerance. We used income per month as a proxy for socio-economic status.

Subjects also reported their level of mobility by selecting 1 of 6 descriptive answers to the question “What is your current moving ability?” [“The response options were “full ambulatory status”, “capable of walking but not running”, “capable of walking 1 km”, “capable of walking 100 m”, “capable of moving only indoors” or “unable to move”]. Women self-reported their physical activity level as hours per week including type and the intensity of physical activity.

In *Study I*, the activity level was compiled from the frequency of physical activity times per week and mobility status. Women were classified as passive if they had restricted or no mobility and exercise ≤ 2 times/week and those with no mobility restriction and exercise > 2 times/week were classified as active.

4.4 Anthropometric measures and body composition

Height and weight of participants were measured in light indoor clothing without shoes, and BMI was calculated kg/m^2 . To measure body composition whole body DXA scans were performed by specially trained nurses, using the same Lunar Prodigy adhering to the imaging and analysis protocols provided by the manufacturer (Lunar Co., Madison, WI, USA) at the baseline and at the 3 years' follow-up. DXA provided measures of bone mass, fat mass and MM. DXA is a widely used tool to estimate the body composition in terms of evaluating the ratio between fat, muscle, and bone in different parts of the body. DXA also has been shown to

be more precise than bioimpedance for estimating body composition (Aandstad et al. 2014, Lohman et al. 2009).

BMC (g) was measured at LS (L2-L4), FN and total body, and BMD (g/cm²) was calculated as BMC (g)/bone area (cm²). Absolute changes in BMD and BMC were further calculated with the use of baseline and year 3 values. Appendicular lean mass (aLM) was calculated as the sum of the nonfat, nonbone skeletal MM in arms and legs. The relative skeletal muscle index (RSMI) was calculated as aLM divided by the square of height (m²). LM index (LMI) and fat mass index (FMI) were calculated as total body LM and FM values divided by square of height (m²). Furthermore, absolute changes in LM, aLM and trunk LM were calculated by subtracting the baseline values from those measured at year 3 (Isanejad et al. 2016, Isanejad et al. 2015). The long-term reproducibility (CV) of the DXA instrument for BMD during the study period, as determined by regular phantom measurements, was 0.4% (Kärkkäinen et al. 2010).

4.5 Physical function

Physical function measures were assessed by trained nurses at the baseline and at the 3 year follow-up session, consisting of three main domains. 1) Muscle strength: hand grip strength (PSI), number of chair rises in 30 seconds, ability to squat, ability to squat to the ground, and knee extension (kg). 2) Mobility test: 10-meter-walking speed m/s) and tandem walk for 6 m (m/s); and 3) Balance ability: standing with closed eyes for 10 seconds and one leg stance performance for 30 seconds.

Grip strength

Grip strength was measured from the nondominant hand while sitting on a bench, with the forearm flexed from the elbow at a 90 angle, near the torso. A total of three attempts were recorded, with approximately 30 s of resting time between the tests. Close attention was paid to make all three attempts in a similar, fixed posture (JAMARTM handgrip dynamometer;

Sammons Preston, Bolingbrook, IL). The best attempt out of the three was recorded as the maximal result (Rikkonen et al. 2012). The intraclass correlation coefficient for grip strength measurements was 0.93. To standardize, grip strength was further expressed as a ratio to body mass (FM+ MM).

Knee extension

Isometric knee extension strength was measured three times from both legs, with a knee flexion of 65 (dynamometer chair; Metitur Oy, Jyväskylä, Finland)(Rikkonen et al. 2012). Participants extended the leg against the ankle strap with maximal effort, and peak force was recorded. Between each maximal attempt, there was approximately 30 s of rest. The sitting posture was fixed straight with an adjustable backrest and tightened hip belt. The ankle strap was individually adjusted to meet the distal end of the lateral malleolus in the performing leg to minimize anthropometric bias. The average of the highest two out of the three attempts per leg was recorded as the maximum score. The results from both legs were then summed up and divided by 2, forming the overall quadriceps strength score used in the analysis. The intraclass correlation coefficients for the right and left knee extension strength measurements were 0.88 and 0.82, respectively.

Chair rise

The chair rise test was conducted if participant could rise at least once without using arms from a straight-backed, non-padded, armless chair. The number of maximum chair rises were recorded by trained nurses.

Walking speed

Women were asked to walk the 10 m distance first normally and then the 6 m in tandem position. Time was recorded and the walking speed was calculated as m/s. The study

population was divided into quartiles according to the 10 m walking speed. The women who were not able to walk were allocated into the lowest quartile.

Short physical performance battery (SPPB)

The SPPB has been suggested to be applicable in clinical practice and studies to evaluate an individual's physical ability (Cruz-Jentoft et al. 2010). The SPPB score was calculated based on EWGSOP definition. Three individual measures of physical function assessments including 10 m walking speed (m/s), chair rises in 30 seconds and one leg stance performance were included. Individuals unable to complete the task received a score of 0, physical function tests were further categorized into quartiles and each quartile was scored on a scale of 1–4 points. The total SPPB score ranged from 0 to 12 with higher scores referring to better performance. Previous studies indicated that an SPPB cut-off point of less than 10 identifies individuals at increased risk of mobility disability. However, due to the low number (8 %) of women with an SPPB score over 10, we defined the development of “mobility disability” as an SPPB score in the lowest quartile (<8.4). There is a link between leg MM and muscle strength (Rennie et al. 2004). The lower body muscle quality (LBMQ) was calculated using 10 m walking speed per leg MM.

4.6 Diagnostics of sarcopenia

Sarcopenia was defined according to EWGSOP criteria (Cruz-Jentoft et al. 2010). Women were categorized into quartiles according to their RSMI values: (1) 5.3–6.3 kg/m², (2) 6.3–6.7 kg/m², (3) 6.7–7.2 kg/m² and (4) 7.2–9.3 kg/m². Baumgartner et al. (Baumgartner 2000) reported that the sarcopenia cutoff point was 5.45 kg/m², which was calculated as two standard deviations below the mean in the young reference population. However, in our study, there were only six women whose RSMI was less than 5.45 kg/m². Therefore, we decided to use the lowest quartile below 6.3 kg/m² as cutoff in the present study. The study population was divided into quartiles also

for their grip strength: (1) < 22.2 kg, (2) 22.3–25.6 kg, (3) 25.7–28.6 kg and (4) >28.7. Physical performance, as assessed by a 10 m walking speed test, was categorized into quartiles: (1) < 1.42 m/s, (2) 1.42–1.63 m/s, (3) 1.64–1.85 m/s and (4) >1.85 m/s. The women who were not able to walk were allocated into the lowest quartile. A woman was classified as sarcopenic if she belonged to the lowest quartile of RSMI and had the lowest quartile of either grip strength or walking speed or both. Pre-sarcopenia was defined if women were in the lowest quartile of RSMI but not in the lowest quartile grip strength and walking speed. Non-classified women belonged to the lowest quartile of either grip strength or walking speed or both, but not to that of RSMI.

4.7 Frailty ascertainment

We used the criteria developed by Fried and colleagues to define frailty (Fried et al. 2001). Frailty has been operationally defined as the presence of three or more of the following criteria: weakness, slowness, weight loss, exhaustion and low physical activity. Frailty status was defined at the 3-year follow-up. In this data, frailty phenotype was defined as the presence of at least three and prefrailty as the presence of one or two of the Fried criteria: low grip strength <22.2 kg, low walking speed <0.51 m/s, low physical activity <3hours/week, weight loss >5% of BW, and exhaustion. The frailty ascertainment is described in Table 9.

Table 9. Frailty ascertainment in elderly women of the OSTPR-FPS population

1- Weakness	To standardize according to Fried criteria (Fried et al. 2001), grip strength was expressed as a ratio to BMI and those in the lowest quartile <22.2 kg were categorized as weak (score=1).
2- Slowness	Walking speed was standardized and adjusted for height according to the Fried recommendation (Fried et al. 2001). Women in the lowest quartile of walking speed < 0.51 m/s were defined as slowest (score=1).
3- Weight loss	The intentionality of the weight loss was not questioned in this study. Therefore, according to the Fried recommendation we used weight loss over 5% of body weight in the previous 3 years as a cutoff (score=1).
4- Exhaustion	<p>Life satisfaction was assessed at 3-year of follow-up using the four-item scale.</p> <p>a) Interest in life (very interesting=1, interesting=2, cannot say=3, boring=4, and very boring=5).</p> <p>b) Happiness in life (very happy=1, happy=2, cannot say=3, unhappy = 4, and very unhappy=5).</p> <p>c) Ease of living (very easy=1, easy=2, cannot say=3, hard = 4, and very hard= 5).</p> <p>d) Feelings of loneliness (not at all lonely=1, cannot say=3, lonely=4, and very lonely=5).</p> <p>Points were summed and ranged 4–20. Points range 12-20 was considered as exhaustion (score=1).</p>
5- Low physical activity	Women were considered having low physical activity if they belonged to the lowest quartile of total physical activity (hours per week) at the 3-year follow-up (score=1).
Frailty score	Points were summed and each participant received a frailty score in a range of 0–5. According to the Fried criteria (Fried et al. 2001); those with score 0 were assessed as normal, score 1–2 as prefrail and more than 3 as frail.

4.8 Statistical analysis

Statistical analyses were performed using the SPSS Statistics for Windows software versions 19 (*Studies I and II*), and 20 (*Studies III-V*) (SPSS Inc., Chicago, IL, USA). All associations and differences were considered statistically significant if the P value was <0.05 . Intervention of vitamin D and calcium might have had an effect on some of the outcomes that were assessed. However, in our analysis, we could find no evidence of any effect of the intervention (vitamin D and calcium supplementation) on the outcomes of interest (BMD, MM, physical function measures, sarcopenia and frailty). Therefore, the whole population was included in the analysis. However, we conducted a stratified analysis with only the control group if the interaction terms between exposures and intervention were significant (*study I*). Furthermore, all prospective analyses were adjusted for intervention group (vitamin D and calcium supplementation) to control for any potential effect of vitamin D on physical performance.

One way ANOVA was used to compare means and standard deviations (SD) between the categories and standard deviations (SD) for continuous variables and Chi-square tests for categorical variables. Independent sample t-test was used to test differences in baseline characteristics of subjects between intervention and control group (*study I-III and V*), sarcopenic and not sarcopenic (*study III and V*), and frail and not frail subjects (*study IV*). The specific statistical analysis methods and models for each study of this doctoral thesis are presented in Table 10.

Selection of potential confounders was a priori based on the literature about their possible effect on the outcomes of interests. Further, we evaluated the effect of covariates on dependent or independent variables in a bivariate correlation analysis, if the association was not significant ($P > 0.10$), they were excluded from the models.

Table 10. Statistical analysis and adjusted covariates of studies I–IV in this doctoral thesis.

Study	Statistical analysis	Independent variable	Adjusted covariates in final model
Study I	Multiple linear regression or logistic regression models	<u>Independent variables:</u> protein intake expressed as g/kg BW ≤ 0.8 , 0.81-1.19, and ≥ 1.2 . <u>Dependent variables:</u> BMD and BMC measures at the baseline and changes in them over 3 years of follow-up.	age, energy intake, height, weight, intervention group, dietary calcium and vitamin D intake, self-reported vitamin D and calcium supplementation, smoking status, physical activity level, hormone therapy use, time since menopause, diseases and use of medications which affect BMD. BMD and BMC variables at the baseline were entered in longitudinal models.
	Multiple linear regression models.	<u>Independent variables:</u> energy-adjusted protein intake (g/d) using residual methods (Willett et al. 1997).	age, height, total energy intake, intervention group, baseline MM measures, smoking, alcohol use, physical activity level, hormone therapy use, and FM.
Study II	Stratified longitudinal analysis between intervention and control groups.	<u>Dependent variables:</u> LM, aLM and trunk LM at the baseline and changes in them over 3 years of follow-up.	
Study III	Multiple linear regression or logistic regression models	<u>Independent variables:</u> Protein g/kg BW ≤ 0.8 , 0.81-1.19, and ≥ 1.2 . In the second set of analysis, LM and FM were introduced as independent variables. <u>Dependent variables:</u> physical function measures.	age, energy intake, smoking status, alcohol consumption, physical activity, hormone therapy use, osteoporosis, LM, height and baseline physical function measures.
Study IV	Multinomial logistic regression models	<u>Independent variables:</u> Protein intake g/kg BW < 1.1 vs. ≥ 1.1 , and in quartiles. BMI ≥ 30 kg/m ² , BMI 25 to < 30 kg/m ² , and BMI 18.5 to < 25 kg. <u>Dependent variables:</u> Frailty according to the Fried criteria.	age, energy intake, intervention group, current smoking, alcohol consumption, hormone therapy use, coronary heart disease, physical activity, and income per month.

Table 10 continued

<i>Study</i> V	Multiple linear regression and logistic regression models.	<u>Independent variables:</u> MED and BSD scores in quartiles. <u>Dependent variables:</u> Sarcopenia, LM, RSMI, and physical function measures.	age, energy intake, smoking, total physical activity, hormone therapy, osteoporosis, rheumatoid arthritis, income per month, and FM percentage.
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BW, body weight; LM, lean mass; aLM, appendicular lean mass; FM, fat mass; RDA, recommend daily allowance; NNR, Nordic Nutrition Recommendation; MED, Mediterranean diet; BSD, Baltic Sea diet; BMI, body mass index; SPPB, short physical performance battery.

5 Results

Table 11 summarizes the hypotheses and main results of *studies I-V*.

Table 11. Hypotheses and results of studies I-V.

Hypothesis	Results
<p><i>Study I</i></p> <p>Higher protein intake has a beneficial association with bone health.</p>	<p>In the cross-sectional analysis, women with protein intake ≥ 1.2 g/kg BW had lower FN BMD, LS BMD and total BMD and BMC (P trend≤ 0.009) compared to those with protein intake < 1.2 g/kg BW. In the follow-up analysis, protein intake (g/kg BW) was inversely associated with LS BMD and LS BMC.</p>
<p><i>Study II, III and IV</i></p> <p>Elderly women with higher protein intake have less LM loss, better physical function and a lower risk of frailty.</p>	<p>At the baseline, women with protein intake ≥ 1.2 g/kg BW had better GS/BM, knee extension/BM, one-leg stance, chair rise, faster 10 m walking speed and higher SPPB compared with those with lower intake, respectively ($P < 0.041$). Total and animal protein intakes were positively associated with LM and trunk LM ($P \leq 0.050$).</p> <p>In follow-up results, higher protein intake was associated with less decline in GS/BM, one-leg stance and tandem walk for 6 m. Also, higher total and animal protein intake were associated with increased LM and ALM ($P \leq 0.050$). Protein intake ≥ 1.1 g/kg BW was associated with lower risk of prefrailty (OR=0.08 and 95% CI=0.01-0.73) and frailty (OR=0.08 and CI=0.01-0.72) compared to those with protein intake < 1.1 g/kg BW.</p>
<p><i>Study V</i></p> <p>A healthy diet indicated by higher BSD and MED score is related to a lower risk of sarcopenia.</p>	<p>Higher BSD and MED scores were associated with greater LM, faster 10 m walking speed, and higher LBMQ (P trend≤ 0.041). Women in the higher quartiles of BSD and MED scores lost less RSMI and total LM over 3-year follow-up (P trend≤ 0.034).</p>

BMD, bone mineral density; BMC, bone mineral content; BW, body weight; FN, femoral neck; LS, lumbar spine; BMI, body mass index; GS, grip strength; BM, body mass; SPPB, short physical performance battery; LM, lean mass; ALM, appendicular lean mass; OR, odds ratio; CI, confidence intervals; RSMI, relative skeletal muscle index; BSD, Baltic Sea diet; MED, Mediterranean diet.

5.1 Baseline characteristics of the participants

The total study population comprised 554 elderly women. The basic characteristics are presented in Table 12 and food and dietary intakes in Table 13. The participants were 65–72 years old (age was 67.8 ± 1.8), mean BMI was 27.4 ± 4.2 kg/m²; according to the WHO criteria, 43.8% (n= 267) of women were overweight, and 24.6 % (n=150) were obese. At the baseline 4.8% of subjects self-reported to smoke, and 46.7 % (n=285) were using hormone therapy. There were no significant differences in baseline characteristics between intervention and control groups.

Mean energy intake was 6560 ± 1556 kJ/d. With respect to the total energy intake, 17.5% was obtained from protein, 49% from carbohydrate, and 31% from fat. Total protein intake was 68.2 g/d which corresponded to 0.96 g/kg BW. The minimum protein intake reported was 0.24 g/kg BW and the maximum 2.25 g/kg BW. Furthermore, 166 (30%) of women had protein intake ≤ 0.8 g/kg BW, 48 % had 0.8-1.19 g/kg BW, while 22% consumed protein ≥ 1.2 g/kg BW. The BSD score ranged from 1 to 25 in our population, and the mean was 13 points. The mean for MED score was 4.7 and ranged from 0 to 8.

During the follow-up of 3 years, FN BMD decreased by -1.89%, while LS and total body BMD increased by +0.93% and +0.56%, respectively. The LM, aLM and trunk LM changes over the three years were +0.69 %, -0.27 % and +0.48 %, respectively. During the 3 years of follow-up, about 24 % of participants lost >3% of their BW, 27% of participants gained >3% of their body weight and 49% were weight-maintainers (within $\pm 3\%$ of baseline weight). Mean changes in aLM were a decrease of 0.57 ± 0.95 kg in weight losers, a decrease of 0.27 ± 0.85 kg in weight maintainers and an increase of 0.19 ± 1.2 kg among weight gainers.

Table 12. Baseline characteristics of the OSTPRE-FPS population.

<i>Study I-V</i>		
	Mean	SD
Age (y)	67.8	1.8
Weight (kg)	72.3	12.2
Height (cm)	158.6	5.3
BMI (kg/m ²)	27.4	4.2
BMI category n (%)		
Normal, 18.5-24.9 kg/m ²	163 (26.7)	
Overweight, 25 to <30 kg/m ²	267 (43.8)	
Obese, ≥ 30 kg/m ²	150 (24.6)	
Smoking, current smoker n (%)	29 (4.8)	
Income per month (euros)	861	294
Physical activity (hours/week)	10.9	15.1
Hormone therapy use n (%)	123 (20.2)	
Sarcopenia status n (%)		
Normal	274 (44.9)	
Presarcopenia	78 (12.8)	
Sarcopenia	202 (42.3)	
Frailty status n (%)		
Normal	312 (52.7)	
Prefrailty	206 (46.8)	
Frailty	36 (5.9)	
Body composition		
Total body lean mass (kg)	40.2	4.3
Total body fat mass (kg)	28.8	8.7
Total body bone mass (kg)	2.2	1.2

BMI, body mass index

Table 13 Dietary factors and nutrient intake (n=554)

	Mean	SD
Energy intake (kJ/d)	6563	1558
Protein intake, crude (g/d)	68.2	18.0
Protein intake energy-adjusted ^a	65.0	11.7
Protein intake (% energy)	17.5	3.1
Protein intake (g/kg BW)	0.96	0.29
Fat intake (g/d)	54.1	18.2
Fat intake (% energy)	31.0	5.6
Carbohydrate intake (g/d)	193.6	48.5
Carbohydrate intake (% energy)	49.0	5.8
Alcohol intake (g/d)	9.9	16
Baltic Sea diet score	12.9	4.0
Mediterranean diet score	4.1	1.3

^a Adjusted using residual method (Willett et al. 1997)

5.2 STUDY I: ASSOCIATION OF PROTEIN INTAKE WITH BONE MINERAL DENSITY AND BONE MINERAL CONTENT

Cross-sectional analysis

At the baseline in the multivariable adjusted linear regression model, energy-adjusted total protein intake ($\beta \geq -0.19$ and $P \leq 0.029$) and intakes of animal protein ($\beta \geq -0.02$ and $P \leq 0.029$) were inversely associated with FN BMD and FN BMC, while no such association was observed for plant protein intake. Further, total protein intake (g/kg BW) ($\beta \geq -0.28$ and $P \leq 0.009$) was inversely associated with FN, LS and total BMD and BMC. Similar findings were observed when using other categories of protein intake (g/kg BW) where those women with a higher protein intake ≥ 1.2 g/kg BW had the lowest LS, FN and total BMD and BMC at the baseline.

Prospective analysis

Tests for interaction for protein intakes (g/d, and g/kg BW) and interventional vitamin D and calcium supplementation were not significant ($P \geq 0.660$). Thus, the whole study population was evaluated in the final analytical model. In the multivariable adjusted model of prospective analysis, total protein intake (g/kg BW) was inversely associated with changes of LS BMD and LS BMC ($\beta \geq -0.30$ and $P \leq 0.002$) (Table 14).

Protein and BMI interaction association with BMD and BMC

The interaction between protein intake and BMI was significant only for the association with FN and LS BMC (P interaction ≤ 0.007). At the baseline, in women with BMI ≤ 30 kg/m², total protein (g/kg BW) was negatively associated with LS, FN and total BMD ($\beta \geq -0.25$ and $P \leq 0.050$) as well as FN and total BMC ($\beta \geq -0.31$ and $P \leq 0.007$). In the prospective analysis, among women with BMI ≤ 30 kg/m², total protein intake (g/kg BW) was negatively associated with a change of LS BMD ($\beta = -0.31$ and $P = 0.016$). No significant association was observed in obese women.

Protein and activity level interaction association with BMD and BMC

The association of total protein intake (g/kg BW) at the baseline and at the 3 year of follow-up was further explored according to the activity level of the participants. The interaction between total protein and activity level was significant only in the association with total BMC and BMD (P interaction ≤ 0.050). At the baseline, the total protein intake (g/kg BW) was negatively associated with FN BMD ($\beta \geq -0.26$ and $P \leq 0.041$) and FN BMC ($\beta \geq -0.22$ and $P \leq 0.036$) in both physically passive and active women. In the prospective analysis, among passive women, the total protein intake (g/kg BW) was negatively associated with LS BMD and LS BMC loss ($\beta \geq -0.43$ and $P \leq 0.003$), while among active women total protein intake (g/kg BW) displayed

positive relationships with changes of LS BMD ($\beta=0.23$ and $P=0.047$) and FN BMC ($\beta=0.21$ and $P=0.049$) over the 3 years of follow-up.

Table 14. Prospective association of protein intake and changes in BMD (g/cm²) and BMC (g).

	FN BMD			LS BMD			Total BMD			FN BMC			LS BMC			Total BMC		
	β	SE	P	β	SE	P	β	SE	P	β	SE	P	β	SE	P	β	SE	P
Total protein (g/d)																		
Model 1	0.07	0.01	0.077	0.05	0.01	0.273	0.11	0.01	0.044	0.08	0.01	0.050	0.07	0.01	0.138	0.03	0.36	0.505
Model 2	0.08	0.01	0.239	-0.03	0.01	0.617	0.12	0.01	0.174	0.10	0.01	0.164	-0.06	0.02	0.420	-0.08	0.58	0.064
Animal protein (g/d)																		
Model 1	0.08	0.01	0.056	0.08	0.01	0.075 [†]	0.11	0.01	0.035	0.09	0.01	0.038	0.07	0.01	0.110	0.04	0.35	0.442
Model 2	0.10	0.01	0.160	0.03	0.01	0.712	0.17	0.01	0.077	0.12	0.01	0.123	-0.04	0.02	0.569	-0.05	0.59	0.531
Plant protein (g/d)																		
Model 1	-0.07	0.01	0.095	-0.10	0.01	0.075	-0.09	0.01	0.070	-0.07	0.01	0.091	-0.05	0.03	0.247	-0.10	0.95	0.053
Model 2	-0.05	0.01	0.301	-0.11	0.01	0.066	-0.14	0.01	0.054	-0.04	0.01	0.409	-0.04	0.04	0.492	-0.08	1.10	0.208
Total protein (g/kg body weight)¹																		
Model 1	0.02	0.01	0.692	-0.14	0.01	0.038	0.05	0.01	0.471	0.09	0.05	0.141	-0.09	0.70	0.168	-0.01	21.12	0.928
Model 2	-0.01	0.01	0.918	-0.31	0.01	0.001	0.04	0.01	0.507	0.16	0.07	0.083	-0.30	1.02	0.002	-0.16	30.04	0.159

BMD, bone mineral density; FN, femoral neck; LS, lumbar spine; SE, standard error.

Model 1 was adjusted for age, total energy intake, height (cm), weight (kg), intervention group and baseline BMD and BMC values.

Model 2 was adjusted for variables in model 1 plus dietary vitamin D, dietary calcium intake, self-reported vitamin D and calcium supplementation, smoking status (current, former and nonsmokers), physical activity level (passive and active), hormone therapy use (never used, used), time since menopause (years); diseases and use of medications which affect BMD.

Models for animal protein were also adjusted for plant protein intake. Models for plant protein were also adjusted for animal protein intake.

¹Body weight was excluded from adjusted variables in analysis using protein as expressed per body weight due to high collinearity. However, the result remained significant even after controlling for body weight.

5.3 STUDY II: ASSOCIATION OF PROTEIN INTAKE WITH MUSCLE MASS

Cross-sectional analysis

At the study baseline, in multivariable adjusted linear regression model, energy-adjusted total protein intake was positively associated with LM, aLM and trunk LM ($\beta \geq 0.05$ and $P \leq 0.014$). Animal protein intake (g/d) was positively associated with LM and trunk LM ($\beta \geq 0.08$ and $P \leq 0.010$). No significant association was observed for plant protein intake except for a nonsignificant association with trunk LM ($\beta = 0.06$ and $P = 0.083$). These results did not materially change after adjusting for FM. In the categorical analyses, women in the higher quartile of intakes of total and animal protein at baseline, but not plant protein, had significantly greater LM, aLM and trunk LM ($P \text{ trend} \leq 0.026$).

Prospective analysis

In multivariable adjusted linear regression model in the total population, total and animal protein intake were positively associated with LM and aLM changes over the 3 years of follow-up ($\beta \geq 0.09$ and $P \leq 0.041$). The intake of plant protein in the total population was positively associated with the aLM change ($\beta = 0.09$ and $P = 0.035$) and non-significantly associated with the LM change ($\beta = 0.09$ and $P \leq 0.056$) over the 3 years of follow-up.

The interactions between energy-adjusted total protein, animal protein and plant protein intakes (g/d) and vitamin D and calcium supplementation were not significant ($P \geq 0.730$). In the intervention group, the energy-adjusted intakes of total protein and animal protein but not plant protein were significantly associated with changes of LM and aLM ($\beta \geq 0.22$, $P = 0.001$) over 3 year of follow-up (Table 15). No significant association was observed in the control group except that plant protein was non-significantly associated with the aLM change ($\beta = 0.11$,

P= 0.082). In follow-up analysis using quartiles of protein intakes in the intervention group women in highest quartiles of total and animal protein intakes had significantly increased LM and aLM (P trend ≤ 0.001) as compared to those in lower quartiles, while no such association was observed for plant protein intake. No significant association was observed in the control group.

Weight change interaction

The association of energy-adjusted total protein intake (g/d) with LM changes were further explored according to whether the participants had undergone a weight change. Weight change and energy-adjusted total protein intake interactions were significant (P interaction < 0.001). Among weight maintainers, but not among weight losers or gainers, the energy-adjusted total protein intake (g/d) was positively associated with a change of LM and aLM and trunk LM ($\beta \geq 0.13$ and $P \leq 0.020$) (Figure 4).

Table 15. Prospective association of protein intake and changes in MM in total population and in intervention and control groups.

	LM (kg)			aLM (kg)			Trunk LM (kg)		
	β	SE	P	β	SE	P	β	SE	P
Total protein (g/d)									
<i>Intervention group (n=270)</i>									
Model 1	0.19	8.52	0.003	0.20	0.01	0.001	0.09	6.05	0.032
Model 2	0.27	8.27	<0.001	0.27	0.01	<0.001	0.15	5.95	0.024
Model 3	0.22	10.70	0.001	0.24	0.01	0.001	0.10	7.60	0.125
<i>Control group (n=282)</i>									
Model 1	-0.03	9.97	0.559	-0.03	0.01	0.539	0.01	5.82	0.955
Model 2	-0.40	9.30	0.538	-0.51	0.01	0.429	0.01	6.05	0.877
Model 3	-0.07	12.80	0.275	-0.05	0.01	0.360	-0.03	8.30	0.548
<i>Total population (n=552)</i>									
Model 1	0.07	0.06	0.115	0.06	0.04	0.098	0.05	0.04	0.276
Model 2	0.09	0.06	0.035	0.08	0.04	0.050	0.08	0.04	0.075
Model 3	0.10	0.01	0.032	0.09	0.04	0.041	0.08	0.04	0.081
Animal protein (g/d)									
<i>Intervention group (n=270)</i>									
Model 1	0.17	8.53	0.004	0.19	0.01	0.001	0.08	6.08	0.068
Model 2	0.27	8.26	<0.001	0.27	0.01	<0.001	0.14	6.95	0.027
Model 3	0.22	10.40	0.001	0.24	0.01	0.001	0.09	7.10	0.151
<i>Control group (n=282)</i>									
Model 1	-0.03	8.96	0.557	-0.04	0.01	0.528	0.01	5.90	0.952
Model 2	-0.04	9.28	0.539	-0.05	0.01	0.430	0.10	6.05	0.874
Model 3	-1.03	11.90	0.301	-0.06	0.07	0.334	-0.03	7.70	0.625
<i>Total population (n=552)</i>									
Model 1	0.06	0.06	0.142	0.07	0.04	0.092	0.05	0.04	0.287
Model 2	0.09	0.06	0.049	0.08	0.04	0.047	0.08	0.04	0.080
Model 3	0.10	0.06	0.037	0.09	0.04	0.037	0.08	0.04	0.088
Plant protein (g/d)									
<i>Intervention group (n=270)</i>									
Model 1	0.09	22.12	0.124	0.09	0.01	0.098	0.05	15.79	0.430
Model 2	0.05	22.01	0.436	0.03	0.01	0.608	0.03	15.84	0.575

Model 3	0.10	0.02	0.137	0.08	0.01	0.182	0.06	0.15	0.360
<i>Control group (n=282)</i>									
Model 1	0.06	24.86	0.323	0.09	0.01	0.126	0.01	16.35	0.935
Model 2	0.09	26.57	0.160	0.11	0.01	0.090	0.03	17.33	0.597
Model 3	0.09	0.02	0.158	0.11	0.01	0.082	0.03	0.17	0.601
<i>Total population (n=552)</i>									
Model 1	-0.81	0.36	0.223	-0.15	0.21	0.019	-0.04	2.4	0.482
Model 2	0.09	0.17	0.063	0.09	0.01	0.039	0.04	1.11	0.327
Model 3	0.09	0.17	0.056	0.09	0.01	0.035	0.04	1.11	0.334

LM, lean mass; aLM, appendicular lean mass; SE, standard error.

Model 1 was adjusted for age, total energy intake and baseline height and intervention group. Model 2 was adjusted for variables in model 1 plus smoking status, alcohol use per week, physical activity level and hormone therapy use. Model 3 was adjusted for variables in model 2 plus change of fat mass.

Models for animal protein were also adjusted for PP intake. Models for plant protein were also adjusted for animal protein intake.

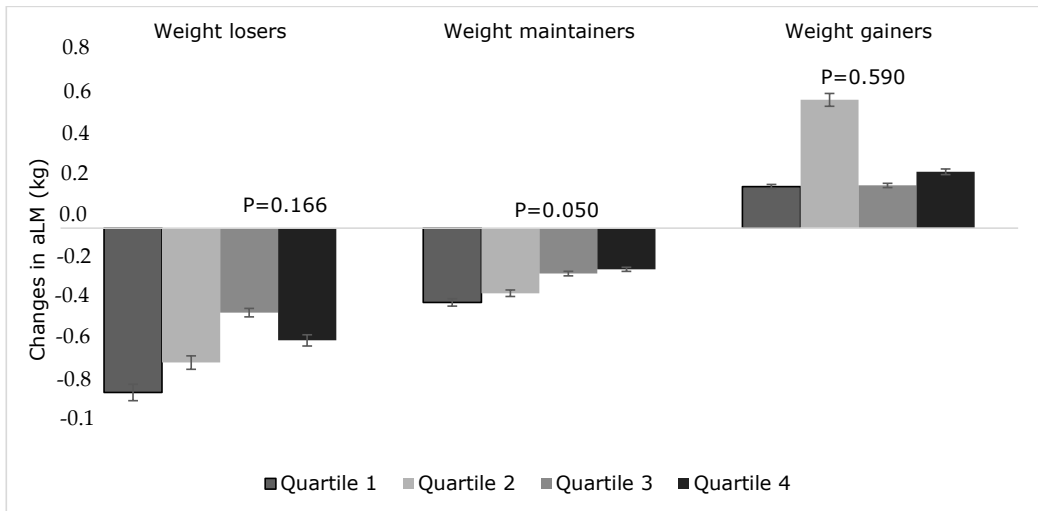


Figure 4. Appendicular lean mass (aLM) change by quartiles of energy-adjusted total protein intake and weight change status, (weight losers n=180, weight maintainers n=278, weight gainers n=96). Adjusted for age, total energy intake, baseline appendicular lean mass, height, smoking status, alcohol use per week, physical activity level, hormone therapy use and intervention group. Tests for a linear trend across quartiles of protein intake within each strata of weight change were conducted by using the median value in each quartile as a continuous variable in the linear regression model. Median total protein intake as a % of total energy intake (g/kg BW) by quartile from quartile 1 to quartile 4 was 14.2% (0.77 g/kg BW), 16.5% (0.89 g/kg BW), 18.5% (0.91 g/kg BW) and 20.1% (1.17 g/kg BW).

5.4 STUDY III: ASSOCIATION OF PROTEIN INTAKE WITH MUSCLE STRENGTH, AND PHYSICAL FUNCTION

Cross-sectional analysis

The sarcopenic group (n= 127) had significantly lower mean weight (-13.2%), BMI (-12.7%), FM (-16.0%) and total body LM (-12.0%) as compared to non-sarcopenic group (n =369). Protein intake was similar in sarcopenic and non-sarcopenic group, 17.6 ± 2.9 % and 17.9 ± 3.1 % of energy, respectively. At the baseline, those with higher protein intake ≥ 1.2 g/kg BW as compared to those with moderate (0.81-1.19 g/kg BW), or lower intake (≤ 0.8 g/kg BW) had greater grip strength/BM (P= 0.001), knee extension force/BW (P= 0.003), longer one leg stance performance (P= 0.047), better chair rise performance (P= 0.043), faster 10 m walking speed (P= 0.005), greater number of squats (P= 0.019) and greater number squat to the ground (P= 0.001), and higher SPPB score (P= 0.004). However, these associations were not statistically significant after further adjustment for FM.

Prospective analysis

Results for the prospective analysis showed that those women with protein intake ≥ 1.2 g/kg BW exhibited a smaller decline in grip strength/BW (P= 0.027), one leg stance performance duration (P= 0.024) and had increased tandem walk speed (P= 0.024), which were no longer significant after controlling for FM. Further analysis indicated that a higher protein intake in non-sarcopenic women displayed a positive relationship with changes of one leg stance performance ($\beta = 0.14$ and P= 0.037) and standing with eyes closed ($\beta = 0.23$ and P= 0.001). No significant associations between protein intake and physical performance measures were observed among sarcopenic women, except for the change in grip strength/BW ($\beta = 0.23$ and

P= 0.037) and a non-significant relation with chair rise change ($\beta = 0.27$ and P= 0.064), which were lost after controlling for selected confounders and FM. Figure 5 shows the grip strength and walking speed across the categories of protein intake.

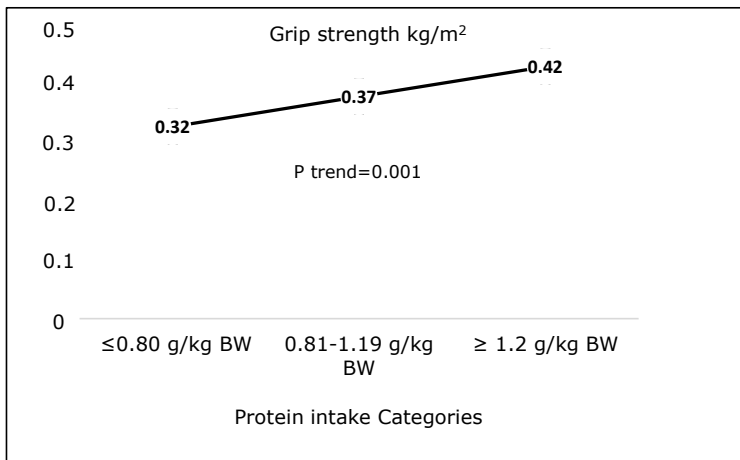
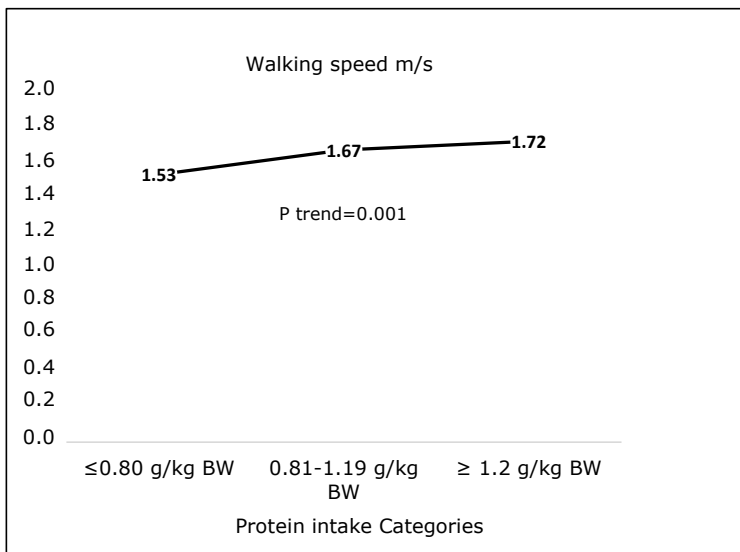


Figure 5. Grip strength (kg/m²) and 10m walking speed m/s across the categories of protein intake g/kg body weight, ≤0.8 g/kg BW (n=171), 0.81-1.19 (n=269), and ≥1.2 (n=170). Tests for a linear trend across categories of protein intake were conducted by using the median value in each quartile as a continuous variable in the linear regression model.



5.5 STUDY IV: ASSOCIATION OF PROTEIN INTAKE WITH FRAILTY STATUS

At the 3 year follow-up, 5.9% (n=36) of women were classified as frail, and 46.8% (n=206) met the criteria for prefrailty. Frailty status was associated with higher BMI and obesity. Protein intake (g/kg BW) and energy intake were lowest in frail women (0.80 g/kg BW), also lower in prefrail women (0.95 g/kg BW) as compared to their normal peers (1.03 g/kg BW). Frail women had a slower walking speed (m/s), lower grip strength (kPa), lower total physical activity (hours/week), they were more often exhausted, but gained weight during follow-up as compared to their counterparts (Table 16).

In multivariable adjusted multinomial logistic regression models, dietary protein intake ≥ 1.1 g/kg BW was associated with lower prevalence of prefrailty (OR=0.08 and CI=0.01–0.73) and frailty (OR=0.08 and CI=0.01–0.72). A 25% increment in protein intake (g/kg BW) was also associated with lower risk of prefrailty (OR=0.09 and CI=0.01–0.90) and frailty (OR=0.07 and CI=0.01–0.67) (Table 17). When we assessed the association of protein intake with components of frailty, it was found that protein intake ≥ 1.1 g/kg BW was associated with lower prevalence of slowness (OR=0.53 and CI=0.28–0.99) and weakness (OR=0.48 and CI=0.26–0.89). Protein intake ≥ 1.1 g/kg BW was not associated with a lower prevalence of low physical activity (OR=0.69 and CI=0.38–1.24).

Table 16. Association of protein intake with frailty status

Models	ORs (95% confidence interval)		
	Normal	Prefrail	Frail
Protein intake ≥ 1.1 g/kg BW vs <1.1 g/kg BW			
Model 1	Reference	0.19 (0.04-0.95) *	0.18 (0.03-0.87) *
Model 2	Reference	0.08 (0.01-0.73) *	0.08 (0.01-0.72) *
Protein intake quartile (g/kg BW) ¹			
Model 1	Reference	0.22 (0.04-1.19)	0.15 (0.03-0.84) *
Model 2	Reference	0.09 (0.01-0.90) *	0.07 (0.01-0.67) *

BW, body weight

*P-value ≤ 0.05

Odds ratios (ORs) derived from multinomial logistic regression models. Model 1 was adjusted for age, energy intake and intervention group. Model 2 was adjusted for Model 1 variables plus smoking, alcohol consumption, hormone therapy use, coronary heart disease, physical activity and income per month.

¹ Tests for a linear trend across protein intake quartiles were conducted by using the median value in each category as a continuous variable in the regression models.

5.5.1 Association of obesity with frailty status

BMI (as a continuous variable) was associated with prefrailty (OR=1.12 and CI=1.06–1.19) and frailty (OR=1.22 and CI=1.11–1.33) (Table 17). Further, obesity was associated with prefrailty (OR=2.81 and CI=1.47–5.37) and frailty status (OR=4.72 and CI=1.26–17.60), while overweight was not associated with prefrailty and frailty. To assess the robustness of the association regarding the possible effect of intervention, we performed a separate analysis for the control group in order to confirm the association of protein intake and obesity with frailty.

Table 17. Association of BMI and obesity status with frailty ¹

	OR (95% confidence interval)	
	Prefrail versus normal	Frail versus nonfrail
BMI (kg/m ²)	1.12 (1.06-1.19)**	1.22 (1.11-1.33)**
BMI category ²		
Overweight (25 to <30 kg/m ²)	1.02 (0.59-1.75)	1.71 (0.43-6.27)
Obese (≥ 30 kg/m ²)	2.81 (1.47-5.37)**	4.72 (1.26-17.60)***

*** *P*-value <0.001, and ***P*-value ≤ 0.05

Odds ratios (ORs) and 95% confidence interval derived from logistic regression models. Model was adjusted for age, energy intake, intervention group, smoking (current smoking), alcohol consumption (g/d), hormone therapy use (yes, no), coronary heart disease (yes, no), physical activity (hours per week) and income per month (euros).

¹ Reference category for prefrail was normal women. Reference category for frailty was nonfrail (normal and prefrail).

² Reference category for BMI was normal (18.5 to <25 kg/m²).

5.6 STUDY V: DIETARY SCORES AND SARCOPENIA

Association of BSD score and sarcopenia indices at the baseline and over 3-year follow-up

At the baseline, in multivariable adjusted regression models, women in the higher quartiles of the BSD score had significantly greater LM (P trend = 0.044), faster 10m walking speed (P trend = 0.006), and a longer one leg stance performance (P trend = 0.050). Those women had also a higher SPPB score (P trend = 0.034) and a better LBMQ (P trend = 0.017). The BSD score was non-significantly associated also with lower mobility-related disability (P trend = 0.051). In the prospective analysis, women in the highest quartile of BSD score lost less RSMI (P trend = 0.022) and total body LM (P trend = 0.015) as compared to those in lower quartiles (Figure 6).

Furthermore, in the analyses using BSD score as a continuous variable, a positive cross-sectional association was observed with total body LM, walking speed, LBMQ and SPPB, whereas the BSD score as a continuous variable was positively associated with proportional changes of RSMI and total body LM over the 3-year follow-up. The interaction of BSD with interventional vitamin D and calcium supplementation was not statistically significant ($P \geq 0.180$). In the stratified prospective analysis in the control group, women in lowest quartile of BSD score lost more RSMI (P trend = 0.018), and total body LM (P trend = 0.004). Women in the highest BSD score quartile, showed the highest SPPB improvement (P trend = 0.041), had a 55% higher squat test completion (OR: 0.45; 95% CI: 0.11-0.98) and a 67% lower risk of sarcopenia (OR: 0.33; 95% CI: 0.13, 0.79) as compared to the lowest quartile, when this was assessed at the 3 year follow-up. Similar results were observed using the BSD score as a continuous variable.

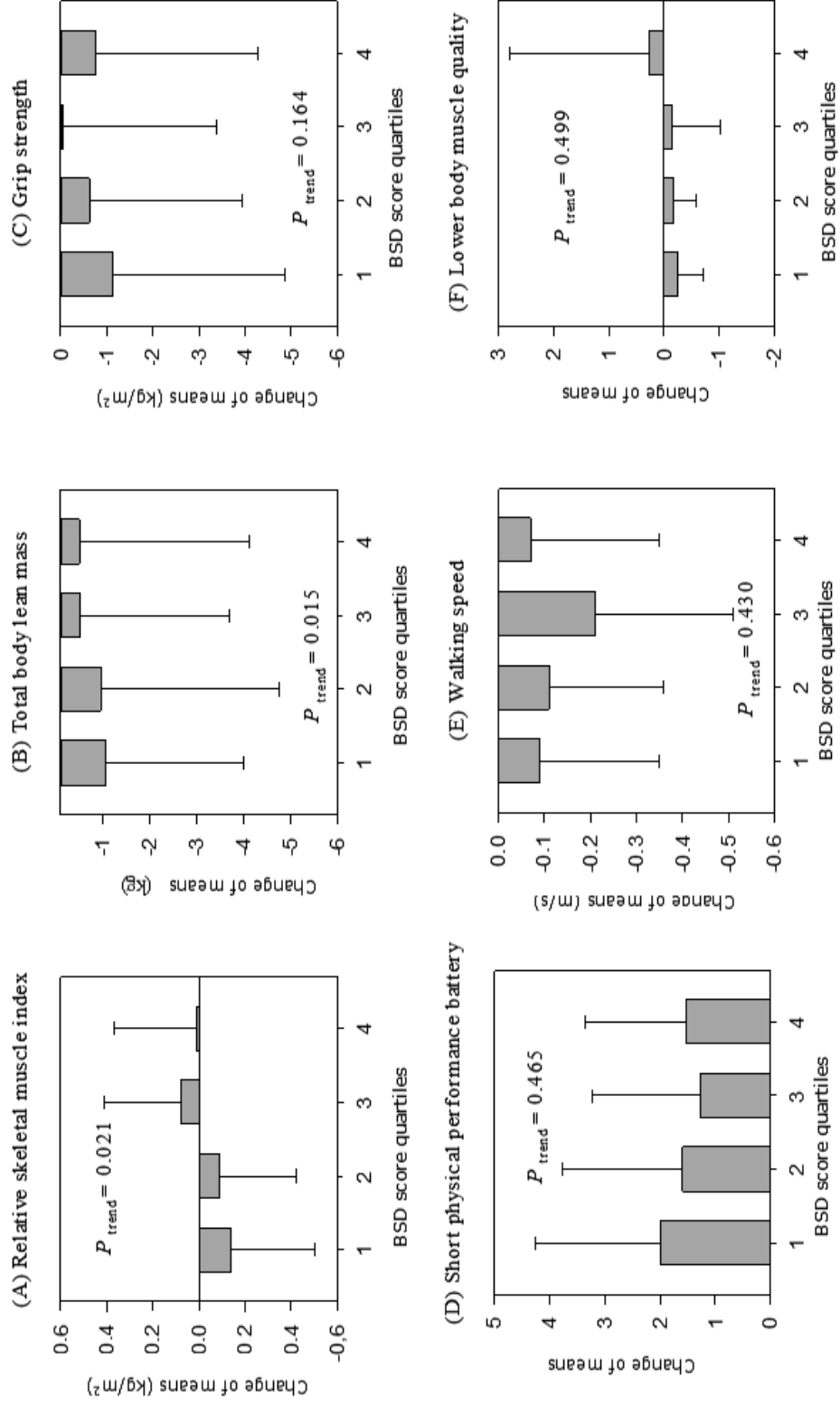


Figure 6. Changes of muscle mass and physical function measures across Baltic Sea diet (BSD) score quartiles. Values are means with their standard errors represented by vertical bars. P trend was based on a linear trend across Baltic Sea diet score quartiles by using the median value in each category as a continuous variable in the linear regression model as exposure. Model was adjusted for age, energy intake, smoking, total physical activity, hormone therapy, osteoporosis, rheumatoid arthritis, coronary heart disease, income per month, fat mass percentage, baseline variables, and intervention group. Median score values in quartiles are 8, 12, 14, and 18.

Association of MED score and sarcopenia indices at the baseline and in the 3-year follow-up

Women in the higher quartiles of MED score had a significantly faster 10 m walking speed (P trend = 0.041), and greater LBMQ (P trend = 0.017) at the baseline. In the prospective analysis, women in the lowest quartile of MED score lost more RSMI (P trend = 0.001) and total body LM (P trend = 0.008) as compared to those in higher quartiles (Figure 7). When using the MED score as a continuous variable, a significant positive association was observed with walking speed and knee extension at the baseline and with proportional changes of RSMI and total body LM over the 3-year follow-up.

Association of BSD and MED score components with sarcopenia indices

In a further analysis, we assessed the associations of the BSD and MED score components with total body LM and physical function at the baseline and over the 3-year follow-up after adjustment for age, energy intake, smoking, total physical activity, hormone therapy, osteoporosis, rheumatoid arthritis, income per month, and FM%. The results detected no significant associations except that higher total fruit and total vegetable (excluding potato) consumptions were positively associated ($\beta \geq 0.08$ and $P \leq 0.049$) with 10 m walking speed, while higher alcohol consumption was negatively associated with 10 m walking speed at the baseline ($\beta = -0.30$ and $P = 0.034$).

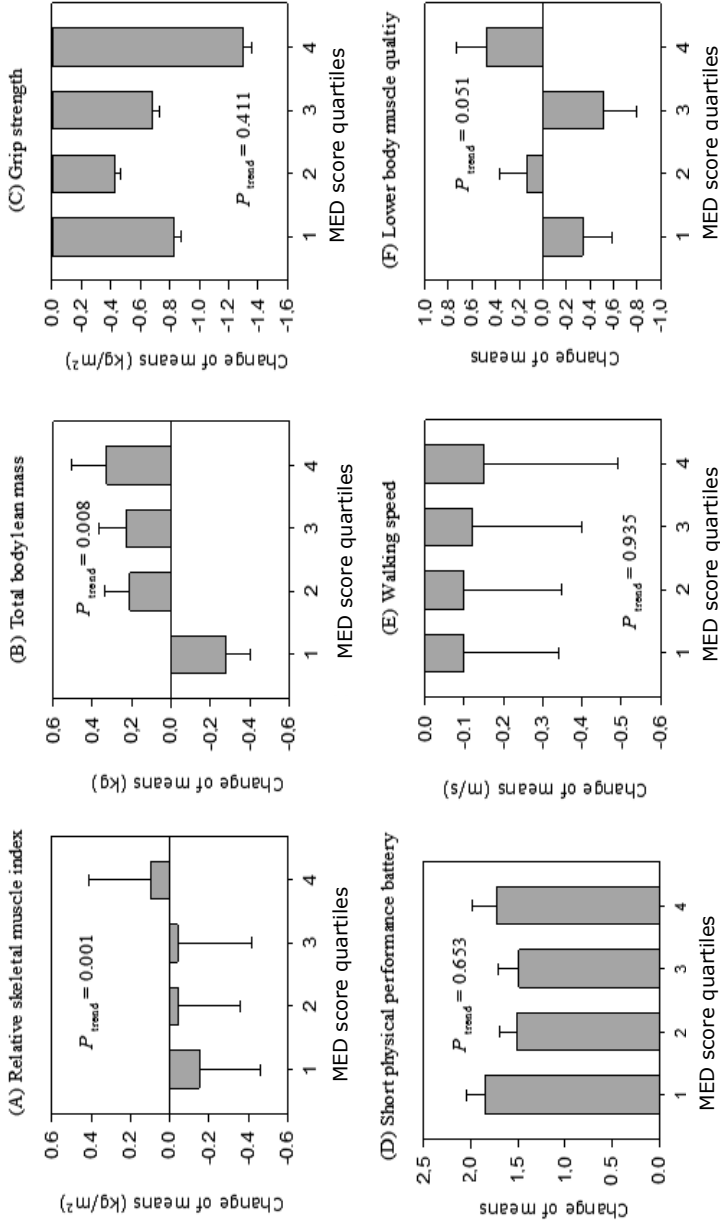


Figure 7. Changes of muscle mass and physical function measures across Mediterranean diet (MED) score quartiles. Values are means with their standard errors represented by vertical bars. P trend was based on a linear trend across Mediterranean diet score quartiles by using the median value in each category as a continuous variable in the linear regression model as exposure. The model was adjusted for age, energy intake, smoking, total physical activity, hormone therapy, osteoporosis, rheumatoid arthritis, coronary heart disease, income per month, fat mass percentage, baseline variables, and intervention group. Median score values in quartiles are 3, 4, 5, and 6.

6 Discussion

This doctoral study examined the association of protein intake with BMD (*Study I*), MM (*Study II*), muscle strength and physical function (*Study III*), frailty (*Study IV*), as well as the association of BSD and MED with sarcopenia (*Study V*). Overall, the results of this doctoral thesis indicated that a higher protein intake in older women might be beneficial for preventing sarcopenia and frailty. However, a higher protein intake was associated with lower BMD in older women. Further, a healthier diet as measured by higher concordance to BSD and MED might reduce the risk of sarcopenia in older women.

6.1 PROTEIN INTAKE AND BONE HEALTH

This study showed that dietary energy-adjusted total and animal protein intakes (g/d), but not plant protein intake, were negatively associated with FN BMD and BMC. In addition, women with a higher protein intake (≥ 1.2 g/kg BW) had lower values of FN, LS and total BMD and BMC. In the prospective analysis, the total protein intake (g/kg BW) was associated with a loss of LS BMD and LS BMC. These findings were observed independently of relevant covariates and confounders. The relation between dietary protein intake and bone health has been studied extensively over the past years (Shams-White et al. 2017). In the recent systematic review conducted by Shams-White et al. (2017) (Shams-White et al. 2017), it was concluded that the role of dietary protein in bone health was unclear/inconclusive, but in their analysis they did not find evidence for any detrimental effects. An adverse association of protein intake and bone health has been hinted at in only a small number of studies (Darling et al. 2011).

The negative association between protein intake and BMD in this study can partially be explained due to strong confounding effect of BMI, physical activity, and calcium+ vitamin D

intakes. Total protein intake (g/kg BW) was negatively associated with BMD and BMC only in women with BMI ≤ 30 kg/m². Although obesity seems to have a positive relationship with BMD, the quality of the bone differs compared to normal-weight individuals (Kanis et al. 2013). Further studies on the interaction of protein intake and BMI on BMD will be required to verify these findings.

Dietary calcium and vitamin D intake as well as supplementation did not have any effect on BMD alone, or in combination with protein intake. It is important to emphasize the limited number of cohort studies examining the possible interaction of protein intake with BMI, physical activity and with calcium + vitamin D supplementation and on bone health, and further studies are clearly merited.

In this study, protein intake was positively associated with changes of LS BMD and FN BMC in physically active women. In a 6-month RCT, 19 healthy early postmenopausal women were allocated to either post-exercise consumption of a protein-containing supplement (with additional calcium and vitamin D) or a placebo supplement (with minimal energy) (Holm et al. 2008). The nutrient-supplement group improved concentric and isokinetic (60°/s) muscle strength from 6 to 24 week by $9 \pm 3\%$, whereas controls did not change. Only the nutrient-supplement group improved MM over the 24 week period of this trial. BMD responded similarly at the LS but changed differently in the two groups at the FN [control: 0.943 ± 0.028 to 0.930 ± 0.024 g/mm³ ($-1.0 \pm 1.4\%$); nutrient-supplement group: 0.953 ± 0.051 to 0.978 ± 0.043 g/mm³ ($3.8 \pm 3.4\%$)].

Furthermore, four cohort studies examined the interaction between protein and calcium + vitamin D on BMD outcomes (Beasley et al. 2014, Dawson-Hughes and Harris 2002, Promislow et al. 2002, Sahni et al. 2014). All except one of the studies (Dawson-Hughes and Harris 2002) did not find evidence for a significant interaction. The results of a cohort study that used data from a 3-year,

RCT (calcium + vitamin D/placebo), showed that only in the supplemented group was higher dietary protein intake associated with less total BMD loss in comparison to those with lower protein intakes (Dawson-Hughes and Harris 2002).

Data of *Study I* showed that the intake of animal protein but not plant protein was negatively associated with FN BMD and BMC. Plant protein based diets contain isoflavones that may have protective effects on bone health. Isoflavones may positively stimulate the osteoblast cell line (favoring proliferation, differentiation and mineralization) and hinder osteoclast and adipocyte generation (Greendale et al. 2015). Animal sources contain more sulphur-containing amino acids such as methionine and cysteine as compared to plant protein sources that can release protons which may decrease the pH and therefore increase the extent of bone dissolution and bone loss (de Jonge et al. 2017).

In addition, higher animal protein intake may increase the risk of atherosclerosis and consequently osteoporosis (Strandberg et al. 2013). In the Nurses' Health Study by Bernstein et al. was shown that greater consumption of red meat or processed meat products was associated with a higher risk of coronary heart disease, while higher intakes of poultry, fish and nuts were associated with lower risk (Bernstein et al. 2010). However, results of a large community cohort (12,066 middle-aged adults, aged 45–64) showed no overall relationship between protein type and major dietary protein sources and risk for coronary heart disease (Haring et al. 2014). Previous epidemiological studies investigating the association of plant and animal protein intakes and BMD have reported inconsistent results (Mangano et al. 2014, Thorpe and Evans 2011). In summary, the results are inconclusive and further research is warranted.

6.2 PROTEIN INTAKE AND MUSCLE MASS AND MUSCLE STRENGTH

This doctoral thesis evaluated the association of protein intake with MM, muscle strength, and physical function as the main outcomes in *Studies II and III*. Their results in conjunction with previous epidemiologic studies suggested that an increased dietary protein intake i.e. higher than recommended for the adult population, can be a beneficial approach to prevent sarcopenia in the older individuals.

The primary finding of this study was that higher intakes of total and animal protein were positively associated with LM and aLM changes over 3 years of follow-up among older women. These results were observed in the vitamin D + calcium supplementation group but not in the control group. In cross-sectional analysis, the energy-adjusted intakes of total and animal protein were positively associated with total body LM and trunk LM, while no such an association was observed for plant protein intake. This is consistent with previous epidemiologic studies, suggesting that a higher protein intake can promote the preservation of MM. In a large prospective cohort study of community-dwelling adults, the Health, Aging, and Body Composition (Health ABC) Study, Houston et al. (Houston et al. 2008) showed that among women aged 70–79 years ($n=2066$), those with higher protein intake (91.0 ± 27.1 g/d) lost 40% less LM as compared to those with lower intake (56.9 ± 18.6 g/ds) over a 3 years' follow-up. Further, Meng et al. (Meng et al. 2009a) found that elderly women with a higher total protein intake (average >1.6 g/kg BW or 20.0 % of energy) had higher LM as compared to those with lower protein intake (average 0.85 g/kg BW or 18.0 % of energy).

In our study, protein intake was associated with LM, aLM and trunk LM changes among those who maintained their weight throughout the 3 years of follow-up but not in those who lost or gained weight. Furthermore, Houston et al. (Houston et al. 2008), showed that a lower protein intake was associated with a greater loss of LM among those who lost weight over the 3-year

period. Thus, recent research efforts have suggested the benefits of increased dietary protein on MM might be more pronounced as a part of an energy controlled diet for maintaining or gaining weight in the older individuals (Paddon-Jones and Leidy 2014).

It is still unknown whether and to what extent vitamin D may influence MM and prevent muscle decline in the older individuals. In 311 men (mean age 56 year) and 356 women (mean age 57 years), no consistent association was found between serum 25(OH)D or PTH and muscle mass, in either men or women (Marantes et al. 2011). A recent meta-analysis of 30 RCTs involving 5615 individuals (mean age 61.1 years) suggested that vitamin D had no significant effect on MM (Beaudart et al. 2014a). The results of the *Study II* did not reveal any direct effect of calcium and vitamin D supplementation on MM, however, the association of intakes of total and animal protein was stronger in those women who received the vitamin D and calcium supplementation. This was in line with a suggested synergic interaction of vitamin D and protein intake to increase MM (Salles et al. 2013, Verreijen et al. 2015). The results of this study are in line with published data and as summarized in this thesis, it seems that a higher protein intake can prevent or slow the decline of MM in the older individuals.

A novel approach of *Study III* was to test the association of protein intake based on NNR 2012 with muscle strength and physical function. The prospective analysis showed that protein intake ≥ 1.2 g/kg BW was associated with a smaller decline in grip strength/BM, and with an improvement in one leg stance performance, as well as the best performance in chair rises in the 3-year follow-up. In the cross-sectional findings, protein intake ≥ 1.2 g/kg BW was associated with physical function measures and SPPB compared to 0.8-1.19 g/kg BW and ≤ 0.8 g/kg BW. However, these associations were no longer significant after adjustment for FM.

Previous published data showed a positive association of dietary protein intake with muscle strength and physical function in the older individuals (Mangano et al. 2017, Sahni et al. 2015,

Lemieux et al. 2014, Gregorio et al. 2014, Geirsdottir et al. 2013a, Bartali et al. 2012b, Morrisand Jacques 2013, Beasley et al. 2013). A 7-year prospective cohort study in the Women's Health Initiative Clinical Trials and Observational Study conducted by Beasley et al.(Beasley et al. 2013) showed that higher calibrated protein intake at baseline was associated with a higher self-reported physical function and a slower rate of functional decline. Women with higher calibrated protein intake also had greater grip strength at baseline and slower declines in grip strength. Women with higher calibrated protein intake completed more chair stands at baseline.

Aging is accompanied by an impaired ability to activate mTORC1 signaling in muscle protein synthesis (23, 64). The loss of efficiency of protein synthesis is thought to be mediated through impairments in mTOR-dependent increases in the initiation of gene translation (64). Furthermore, it has been suggested that post-absorptive muscle protein synthesis may explain the gradual aging decline of MM (69). This kind of imbalance in the older individuals may be combatted by increasing the dietary protein intake as the most important dietary stimuli.

A new aspect of this study was that it provided of evidence that different types of association exist when the relationship between protein intake and muscle strength is studied via the sarcopenia status. Declines in MM might predict a reduction in muscle force and performance. It has not been studied whether a higher MM value is related to anabolic muscle function due to protein intake.

Does protein quality matter?

Previous studies that have separately analysed animal protein and plant protein have yielded conflicting results regarding their associations with MM and muscle strength (Sahni et al. 2015). Although it is recommended that adults have adequate protein intakes for musculoskeletal health, it remains unclear if specific protein food sources or dietary patterns confer greater benefits to the musculoskeletal system. The anabolic potential and quality of protein is

dependent on its essential amino acid profile, digestibility, and amino acid bioavailability (Volpi et al. 2003, Paddon-Jones et al. 2015). It has been suggested that dietary protein intake from animal protein sources due to higher amounts of essential amino acids such as leucine may be relatively more beneficial to prevent sarcopenia and frailty (Volpi et al. 2003). However, the relationship between animal and plant-derived proteins with MM or function has been compared in only a few studies (Haub et al. 2002, Campbell et al. 1999). In the study of Sahni et al. investigating men and women aged 59 years, the total protein intake was 80 ± 26 g/d in men and 76 ± 26 g/d in women (Sahni et al. 2015). In men and women, leg LM was higher in participants in the highest quartile of total and animal protein intake compared with those in the lowest quartiles of intake. Plant protein intake was not associated with lean mass in either sex.

Although plant-based diets are low in certain essential amino acids, they have been linked with higher LM and muscle strength (Pedersen et al. 2013, Sahni et al. 2015). The results of the Framingham Offspring Cohort showed that a higher intake of plant protein was positively associated with quadriceps strength, whereas animal protein intake showed no significant association (Sahni et al. 2015). The results of a recent study suggest that in a population of middle-aged adults, higher intake of dietary protein is linked with LM and muscle strength, but those foods with higher protein contents do not totally account for the associations with these measures of muscle health. One possible explanation may be that the influence of dietary protein on the musculoskeletal system is dependent on many other dietary factors e, g. calcium, magnesium, and vitamin D (Massey 2003).

6.3 PROTEIN INTAKE, OBESITY and FRAILITY

The main findings of this study suggested that protein intake ≥ 1.1 g/kg BW was associated with a lower rate of prefrailty, frailty, slowness and weakness in elderly women. Furthermore,

obesity was strongly associated with higher risk of prefrailty and frailty in elderly women. These associations remained significant even after adjustment for multiple confounders.

Protein intake and frailty

There are very few observational studies using protein intake reflecting on nutrient recommendations. As far as we are aware, there are no studies that have investigated whether the NNR protein intake of ≥ 1.1 g/kg BW would be associated with frailty status. The results of *Study IV* are in line with the growing amount of evidence suggesting that a higher protein intake can prevent or delay the onset of mobility disability and frailty in the older individuals. In the French Three-City cohort among 1345 community dwelling, older adults aged 65 years and older, protein intake ≥ 1 g/kg BW was associated with a lower prevalence of frailty defined by the Fried criteria, and slowness indicated by low walking speed (Rahi et al. 2016). Furthermore, among 2108 grandmothers or acquaintances of dietetic students aged 65 years and older in Japan, total protein intake was significantly inversely associated with frailty. Subjects categorized to the third, fourth, and fifth quintiles of total protein intake (>69.8 g/d) showed significantly lower ORs for becoming frail in comparison to those in the first quintile (Kobayashi et al. 2013). The results of a large longitudinal analysis by Beasley et al. (Beasley et al. 2010) in the Women's Health Initiative study showed that a 20% greater protein intake (% of energy) (the mean protein intake was 1.2 g/kg BW) was associated with a 9% lower risk of prefrailty and a 12 % lower risk of frailty. Furthermore, in CHIANTI study, the prevalence of frailty in older adults in the lowest quintile of protein intake was doubled that of those in the highest quintile. Thus, the available data, although limited, does suggest that an increased dietary protein intake in older adults can substantially decrease the risk for frailty in the older individuals (Dickinson et al. 2013).

Obesity and frailty

The appearance of fat within muscles can affect both muscle strength and muscle function (Visser et al. 1998). In older adult men and women, low MM and high fat infiltration into the muscle have been linked with decreased strength and increased risk of losing mobility. This might be due to a decreased level of physical activity in the obese. Obesity is a common aging problem; it appears to predispose older people to a risk of frailty and mobility disability. In the Helsinki Businessmen Study in 1974, 1815 initially healthy men (mean age 47 years) by Strandberg et al. (Stenholm et al. 2014) was shown that the development of frailty was significantly higher among participants with overweight (BMI ≤ 25 – 30 kg/m²) or obesity (BMI >30 kg/m²) compared with those with normal weight (BMI <25 kg/m²). Cross-sectional analysis in the Women's Health Initiative study (559 women aged 70–79 years) has shown that being overweight was associated with prefrailty, and obesity was associated with both prefrailty and frailty (Blaum et al. 2005). Data from an investigation of 1,119 men and women aged 30 or older (population-based Mini-Finland Health Examination Survey) showed that people with overweight status at the baseline had an increased risk of prefrailty and frailty at the 22-year follow-up when compared to normal-weight individuals (Stenholm et al. 2014). Thus, obesity is one of the underlying causes of frailty and its effects may accumulate already in midlife. Our results indicated that obesity (BMI ≥ 30 kg/m²) but not overweight (BMI 25 to <30 kg/m²) was associated with a higher prevalence of prefrailty and frailty.

6.4 DIETARY PATTERNS AND SARCOPENIA INDICES

As far as is known, this is the first cohort study which has evaluated that association of BSD and MED with extensive measures of sarcopenia. The prospective data showed that women with lower BSD and MED scores experienced a larger decline in RSMI and total body LM as compared to those with higher scores over the 3-year follow-up. The cross-sectional results

showed that a higher BSD score was associated with greater total body LM, faster 10 m walking speed, longer one leg stance performance, higher SPPB score, and greater LBMQ at the baseline. Those with higher concordance to BSD tended to have lower risk of mobility disability. Further, women with higher MED score had faster 10 m walking speeds and greater LBMQ at the baseline. One explanation for the attenuation observed in the prospective analysis could be the small changes in physical function measures that occurred over the 3-year follow-up.

Our results were concordant with previously published, although limited, data. In the recent prospective study among ageing women and men, a higher concordance to a healthy Nordic diet (similar to BSD) was associated with better physical performance 10 years later, including the 6-min walk, arm curl and chair stand tests, reflecting better aerobic endurance and upper- and lower-body strength (Perala et al. 2016). The results of the InCHIANTI study (Milaneschi et al. 2011), indicated that a higher concordance to MED was associated with better lower body performance. Participants with higher concordance experienced a lesser decline in the SPPB score, at the 3, 6 and 9 year follow-up, compared to those with lower concordance. A higher concordance to MED at baseline was also associated with a lower risk of low physical activity and low walking speed, but not with feelings of exhaustion and poor muscle strength. In the study of Shahar et al. (Shahar et al. 2012) among 2225 well-functioning men and women aged ≥ 70 years followed-up for over 8 years, both usual and rapid 20 m walking speed declined in the three MED concordant groups; however, the group with the highest concordance to the MED performed better at all the time points. Thus, the current literature accompanied with the results emerging from *Study V* suggest that a healthy diet, especially MED or BSD, may enhance physical performance as well as preventing sarcopenia in the older individuals.

A healthier diet, such as BSD and MED, contains several components which might be beneficial in the prevention of sarcopenia in the older individuals. Data from *Study V* showed that the

intakes of carotenoids, vitamin E and vitamin C were greatest in the highest quartiles of BSD and MED score; a high intake of fruits and vegetables in MED and BSD could provide such antioxidants. Carotenoids and antioxidants quench free radicals; this reduces the damage from reactive oxygen species. They may also modulate redox-sensitive transcription factors (such as NF- κ B, IL-6 and other proinflammatory cytokines) (Rondanelli et al. 2015). Recent epidemiological studies in community-dwelling older adults show that low serum/plasma levels of carotenoids and vitamin E are independently associated with low skeletal muscle strength and the development of walking disability (Semba et al. 2007, Bartali et al. 2006).

In addition, dietary fat quality might have a role in promoting inflammation and therefore sarcopenia in the older individuals. It has recently been demonstrated that inflammation is an important predisposing factor for sarcopenia (Wang et al. 2017). Circulatory cytokines participate in activating or blocking signaling pathways, thus affecting protein synthesis and proteolysis. Both MED and BSD are characterized by high PUFA and MUFA intakes with respect to the SFA ratio. An adequate intake of n-3 PUFA (alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid), a compound purported to possess anti-inflammatory properties, was related to leg strength and chair-rise capacity (Robinson et al. 2008), and similarly consumption of fatty fish (as an enriched source of long chain n-3 PUFAs) has been associated with grip strength (Smith et al. 2011). N-3 PUFAs have the potential to stimulate the muscle protein synthesis and subsequently MM production (Abbatecola et al. 2009). In the InCHIANTI study, after adjustment for potential confounders, baseline n-3 PUFA levels were inversely related to the risk of developing a decline in SPPB (OR= 0.21), while the n-6/n-3 ratio was associated with a higher risk of SPPB decline (OR= 5.23) (Abbatecola et al. 2009). N-3 PUFA plasma levels, which most likely reflect dietary intake, seem to confer protection against the accelerated decline of physical performance. A higher n-6/n-3 ratio was

associated with a higher risk of developing poor physical performance and slower walking speed.

The results of our study have encouraging public health relevance i.e. a higher concordance to BSD and MED might be beneficial in prevention of sarcopenia, since women with lower concordance to BSD and MED lost more RSMI and total body LM compared to others. In addition, in the cross-sectional setting, BSD and MED were associated with better physical function. This highlights the importance of the whole diet, not only single foods or nutrients. Further longitudinal studies are warranted to verify these interesting findings.

6.5 STRENGTH AND LIMITATIONS

6.5.1 Study population and study design

A limitation of this study was that it included relatively healthy elderly women from a rather homogenous Finnish population, so generalization to the whole elderly population needs to be conducted with caution. Our results were obtained in the study participants that were slightly younger than other studies in this field and thus the prevalence of sarcopenia and frailty status were somewhat lower. Participants who took part in an osteoporosis study may have had a heightened awareness of their bone health. This may have led them to alter some of their modifiable osteoporosis risk factors between the baseline and follow-up visits. However, such an effect is unlikely to have influenced protein consumption since protein is not commonly perceived to be an osteoporosis risk factor. In addition, because of observational nature of our study, we cannot establish a causal relationship.

6.5.2 Assessments

Diet

A single 3-day dietary record at the baseline might not be the most reliable method to capture the long-term intake of nutrients and protein intake and FFQ would provide a better predictor of long-term food consumption. Furthermore, food records were collected on consecutive days rather than non-consecutive days, which may reduce day-to-day variability. However, the 3-day food records method has been described as a suitable instrument for assessing energy and protein intake in elderly people (Paddon-Jones et al. 2006, Luhrmann et al. 1999). Therefore, the food record method is believed to provide representative information on the intakes of nutrients in the older individuals. An important strength of this doctoral thesis was that the protein intake was analysed in categories based on the newer intake recommendations for the older individuals (Nordic Nutrition Recommendations 2013), which have not been applied in most of the previous studies.

The 3-day food record and alcohol questionnaire were used to define BSD and MED scores. Most of the components of BSD and MED scores were available in the food record; in only one case and due to a limitation inherent in the software, it was not possible to calculate the red meat intake separately from chicken. Thus, we only included the data on sausage and processed meat intakes.

Body composition

In this data FM, LM and bone mass were measured by DXA; the DXA measurements were carried out using the same Lunar Prodigy, adhering to the imaging and analysis protocols provided by the manufacturer (Lunar Co.) (Kärkkäinen et al. 2010). DXA is currently a commonly used tool suitable for the estimation of body composition in terms of evaluating the

ratio between fat, muscle and bone in different parts of the body (Aandstad et al. 2014). DXA has also been shown to be more reliable than bioimpedance when estimating the body composition (Aandstad et al. 2014). The availability of each full body composition measures at the baseline as well as over a 3 year period added a significant strength to our study.

It could be argued that the observed results could be confounded by instrumental errors. Excess adiposity in obese subjects may artificially decrease BMD, especially in axial sites. In addition, FM loss during weight loss can affect tissue thickness and bone area measurements. In an attempt to minimize these kinds of errors, the present study reported both BMD and BMC, however, particularly for obese individuals, additional efforts to use peripheral bone measurements are required. This study adjusted for body size which is an important modifying factor for LM, by taking into account the baseline height.

Physical function

The availability of multiple standardized physical performance measures at baseline and over a 3-year period added a significant strength to our study. Dynamometric grip strength measurement is an important indicator of both upper and lower limb strength. In addition, knee extension is involved in a variety of functional tasks, such as walking, chair rising and stair climbing. Thus, grip strength and knee extension are particularly enlightening in the quantification of physical performance in older adults.

Sarcopenia diagnostics

We used the EWGSOP criteria to define sarcopenia; this is the most commonly used approach (Cruz-Jentoft et al. 2010). Because of differences in the study population, the cut-off values could not be identical to those recommended by EWGSOP. Baumgartner et al. (Baumgartner 2000) reported that the sarcopenia cut-off point was $\text{RSMI} < 5.45 \text{ kg/m}^2$, which was calculated as two

standard deviations below the mean in the young reference population. However, only six of our participants had an RSMI which was less than 5.45 kg/m². Accordingly, we decided to use the lowest quartile below 6.3 kg/m² as the cut-off in the present study. Furthermore, to achieve balanced numbers of participants in the stratified analysis, women were classified as sarcopenic if they belonged to pre-sarcopenia, sarcopenia and severe sarcopenia (lowest quartile of RSMI) whereas the non-sarcopenic group was compiled from normal and non-classified groups (normal RSMI). However, the arbitrariness of these cut-off points must be acknowledged when interpreting the results.

Frailty ascertainment

Widely accepted criteria for the definition of frailty are lacking. To define frailty we used similar or surrogates of criteria as developed by Fried and colleagues (Fried et al. 2001). Since the calculation of weight change was possible only by subtracting the baseline value from the one at 3 year; frailty status was defined using the measurements at the 3-year follow-up. Thus, studying frailty and diet at the same time point was not feasible in this study.

In this study, the prevalence of frailty (5.9 %) was relatively low, however, prefrailty (46.8 %) was highly prevalent and it was associated with several adverse health outcomes such as obesity. The proportion of frailty and prefrailty in our study was similar to that described by Rahi et al. (Rahi et al. 2016), in a community-dwelling older adults aged 65 and over (n=1345), of whom 55 (4.1%) were identified as frail.

Other assessments

We examined a wide selection for several known confounders that might influence sarcopenia, frailty, and bone density. However, lifestyle factors such as health status, development of disease, some uncaptured aspects in habitual physical activity level and/or dietary habits in

participants might have affected the observed results. For example, the lower protein intake and concordance to healthy dietary patterns might be linked to the inferior health of a participant and since we do not have information on the participants' earlier health status and eating patterns, reverse causality is possible. For instance, those with a higher BSD score were more physically active than those with a lower BSD score.

7 *Conclusions*

The following conclusions can be deduced from the population study on the association of protein intake and dietary patterns with musculoskeletal health.

1. The findings of this study suggest that different measures of protein intake (g/d and g/kg BW) were negatively associated with BMD and BMC.
2. It was observed that higher intakes of total and animal protein were associated with the preservation of LM. It was also noted that the associations between intakes of total and animal protein with increased LM were more apparent among older women who maintained their weight and received vitamin D and calcium supplementation.
3. The results of this cohort study suggest that a higher protein intake might be positively associated with preserving muscle strength and physical function. Thus it is appropriate to focus on the relationship between protein intake with muscle strength and physical function in the older individuals, because this group is rather vulnerable to suffer nutritional deficiencies.
4. This study showed that a higher protein intake and not being obese can be important strategies to prevent the onset of prefrailty and frailty in aging population.
5. A higher concordance to BSD and MED might be beneficial in the prevention of sarcopenia, since women with lower concordance to BSD and MED lost more RSMI and total body LM compared to those with better adherence to these diets.

8 Recommendations

Changes in body composition, sarcopenia and frailty in the older individuals are adverse outcomes of a combination of genetic predisposition and multiple environmental and behavioral factors. Diet is one of the most important modifiable factors for these geriatric conditions. Dietary protein intake is considered as a key nutrient in the development of sarcopenia and frailty. Nonetheless, it is challenging to define the optimal protein intake to lower the risk of sarcopenia and frailty in the older individuals. Most of the previous studies, although limited, have mainly used study population-based cut-off points, while recommendations are set as g/kg BW. The results of this doctoral thesis suggest that protein intake following NNR (2012) can be a practical reference cut-off value in the prevention of loss of MM and physical function decline in the older individuals. Further, it was found that higher protein intake was associated with a lower bone density. However, the effect of BW and physical activity should be considered as a basis for future research for examining how lifestyle factors interact with protein intake.

In addition, this doctoral thesis suggests that studying the separate association of protein intake from a different food source (animal *vs.* plant) with bone and muscle may be inconclusive. Thus, currently the optimal strategy is to encourage older adults to meet required protein intakes rather than to consume specific protein-containing foods. The priority should be given to increasing the dietary protein intake, regardless of the food source; this will likely make it possible to achieve the required amounts in a population in whom adequate energy intake is already a problem.

Very little is known about the effect of other nutrients on sarcopenia and frailty. For instance, dietary patterns can represent a sensible approach to exploring the role of the whole diet on muscle and bone health. The present study contributes to the limited number of studies on dietary patterns associated with the physical function and sarcopenia. This doctoral thesis delivers an encouraging public health message that concordance to healthy BSD and MED can prevent sarcopenia in the older individuals. These dietary recommendations are easily applicable in nutrition recommendation guidelines. Therefore, future researchers can study larger population based samples to clarify the possible effects of diet on sarcopenia and frailty in the older individuals.

Further research is strongly recommended to examine the effect of protein intake and diet as well as obesity and physical activity on sarcopenia and frailty in the older individuals, especially with longer follow-up periods. In addition, the biological and cellular mechanisms behind the observed associations of protein and dietary factors with body composition and physical function are unclear and should be clarified. Clearly, more studies on the effects of dietary protein interventions on the older individuals in risk of sarcopenia and frailty are warranted.

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MASOUD ISANEJAD

Sarcopenia is loss of skeletal muscle mass and strength, which is major cause of frailty and loss of independence in older people.

This study examined the association of protein intake and dietary patterns with musculoskeletal indices among older people.

It showed that higher dietary protein and a healthy diet might prevent sarcopenia in older women. Preventing sarcopenia, via modifiable

behavioral factors such as diet, are of increasing research and public health interest.



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